(2)

9(2)

MEMOIRS
OF THE
FACULTY OF FISHERIES
HOKKAIDO UNIVERSITY

Vol. 9, No. 2

THE FACULTY OF FISHERIES, HOKKAIDO UNIVERSITY
HAKODATE, JAPAN
1961

MEMOIRS
OF THE
FACULTY OF FISHERIES HOKKAIDO UNIVERSITY

Vol. 9, No. 2

THE FACULTY OF FISHERIES, HOKKAIDO UNIVERSITY
HAKODATE, JAPAN
1961

STUDIES ON BOUND WATER IN FISH MUSCLE

MINORU AKIBA

STUDIES ON BOUND WATER IN FISH MUSCLE

By

MINORU AKIBA

Faculty of Fisheries, Hokkaido University, Hakodate, Japan

CONTENTS

I. INTRODUCTION

There are three definitions of Bound Water, as stated in the earlier Paper[1a], such as biological, colloidal and molecular theoretical Bound Water, which have been studied from the standpoints of biology, colloidal chemistry and molecular theory respectively. There are many methods for estimating the amount of Bound Water, *e.g.*, chemical, thermodynamic, electro-chemical *etc.*, but some of them adopt different ideas as to the principle of the estimating method[2c].

Studies on Bound Water were first made about the state of water frozen in various substances. For example, Thoenes[3], Hardy[4], Moran[5], Kinoshita[6] and Kistler[7] have observed the amount of unfrozen water in the meat of dogs and other animals, gelatine, myogen, egg-albumin, *etc.* From the physiological and biological standpoint, Plank[8] has advocated his so-called freezing phase; he has divided into four phases the freezing process of living things within the temperature range from −1°C to −60°C, and has pointed out the existence of biological Bound Water and colloidal Bound Water. Beside the above described studies, Heiss[9] and Joslyn[10] have discussed the relation between the amount of Bound Water and the change of colloidal properties during the refrigeration of animal kingdom foods.

Furthermore, there have been many studies on the hydrophilic colloid of vegetable juice from many years ago[11].

On the contrary, there have been published only a few studies on Bound Water in muscle of marine animals. Higuchi[12] has studied on the variation of

the amount of Bound Water of squid and flat fish meat in the course of drying. Kawakami[13)], using carp muscle, has assumed the existence of Bound Water in fish muscle from his study on the relation between the depression of freezing point and the variation of water-content in the course of drying.

In the earlier Paper I[1a)], the present author has studied on the amount of Bound Water in fish muscle and gelatine by the cobaltous chloride method[14)], the vapour tension method and the method by electrical resistance at 50 cycles. The results obtained were compared with each other and the following conclusions were reached.

(1) In the case of estimation by the cobaltous chloride methods (Hatschek's[14a)] and Oyagi's[14b)]), the author was able to estimate both the amount of Bound Water at the point of B.P. (the blue turning point of dyed sample) and C.P. (the point of apparent constant weight in the drying at 30°C) on the basis of ideas as to the nature of Bound Water. The author has called the water-content at B.P. the maximum amount of Bound Water and that at C.P. the minimum amount of Bound Water.

(2) When the amount of Bound Water in fish meat and gelatine was estimated by the cobaltous chloride methods, and these results were compared with the values on the curve of water-content—water-activity (or relative vapour pressure) obtained by the vapour tension method, the amounts of Bound Water (gm) per gm of the dried matter (free from cobaltous chloride) of the samples at the points of H.B.P. and H.C.P. by Hatschek's and O.C.P. by Oyagi's method were ascertained to be the water-content corresponding to above 0.8~0.7 of the water-activity, "a". These values will give the probable value which has been considered before as the amount of colloidal Bound Water. The amounts of Bound Water (gm) per gm of the dried matter (containing cobaltous chloride) at the points of O.B.P' and O.C.P' will give also the value of the amount of colloidal Bound Water.

(3) In this cobaltous chloride method, cobaltous chloride which was added to the sample in Hatschek's or which penetrated into the sample in Oyagi's method decreases the activity of water in the sample. The degree of decreasing of the water-activity is different from the activity of water in the original sample. That is to say, it is different from the binding strength of water in the sample. In this case, the hydration of cobaltous chloride with water in the sample is restricted in accordance with the increasing of binding strength of water in the original sample.

(4) In the results of the estimation of the amount of Bound Water, the electrical resistance decreases in accordance with the decreasing of water-content, but it begins increase at about 50% of total water-content. The electrical resistance gradually increases at the point of B.P. which was considered to be shown as the maximum amount of Bound Water (the total water-content, 50~30%) by the cobaltous chloride method, and it increases rapidly at the point of C.P. which was considered to be the minimum amount of Bound Water (about 30~20% of total water-content) by the same method. At below 20% of the water-content, the relation between the increasing of electrical resistance and the water-content showed a logarithmic straight line.

(5) Oyagi's method gives a greater amount of Bound Water than Hatschek's method. In an earlier Paper II[1b], the author has discussed the thermodynamic properties of water below 0.7 of "*a*" for the curves of the water-content "*g*"—water activity "*a*" for the common squid meat protein, cod meat protein, raw Atka mackerel meat and gelatine, which were determined by the vapour tension method at two different temperatures. The water-content—water-activity curve was discussed by Katz[15], Moran[5], Brooks[16] and Briggs[17] and recently by Higashi and Nukazawa[2c], but it is difficult to decide what range of the curve indicates the boundary of Free and Bound Water, because the ideas respecting Bound Water are different from the standpoints of biology, colloidal chemistry and molecular theory respectively. However, as a characteristic of the curve of S shape, the water-content—water-activity curve has generally three parts, A, B, and C. The part of B is a gentle grade and its range is 0.2~0.7 of the value of "*a*", that is to say, the variation of the water-content of the sample is small in the range of that water-activity. The parts A and C are steep grade; the range of part A is 1.0~0.7 of the values of "*a*" and that of part C is 0.2~0.0 of "*a*".

In the discussion of Bound Water from the standpoint of molecular theory according to Herrmann, Gerngross and Abits[18] and Katz and Derksen[19] who have examined independently the molecular constitution of gelatine from the X-ray diffraction, and Sponsler, Bath and Ellis[20] who have examined the hydration of amino acids which are components of gelatine, it is considered that 0.5 gm of the water-content per gm of dried gelatine is at least necessary to have a stable constitution of gelatine molecules. According to Adair and Callow[21], the amount of Bound Water is independent of the water-activity, and it takes a definite value of 0.5 per gm of dried matter. Briggs[17] stated that when the water-activity "*a*" is below 0.8~0.7, water corresponding to that water-activity does not act as a solvent. Recently Bull[22] has examined the relation of water-content and water-activity of gelatine and other various high molecular compounds, and analyzed his results by the question of B.E.T.[23a]. He has considered the water of which the activity is below 0.7 of "*a*", is the molecular theoretical Bound Water. Therefore, the present author has analyzed the thermal change in the water content "*g*"—water activity "*a*" curve obtained by vapour tension method by using the above stated samples. He has analyzed the same curve by the equation of B.E.T. of Brunauer *et al.*[23a] in order to learn whether the molecular theoretical Bound Water of protein is constituted of the molecules of water combined with the protein molecule through the hydrogen bond formation in a definite combining potential or not. From his experimental results, the author has ascertained that water below 0.7 of "*a*" in the curve of "*g–a*" should be the so-called molecular theoretical Bound Water on the basis of the idea of hydrogen bond formation.

In the present paper, the author has estimated the amount of Bound Water in raw fresh meat, dried meat, salted meat, fermented meat and decomposed meat of various kinds of fish by the cobaltous chloride method and by the electrical resistance method, and the results obtained will be described in following Section II.

Next, the author has studied the following subjects, and the results obtained were described in separated Sections:

Section III. The influence of Bound Water upon the drying velocity of fish meat.

Section IV. On the relation between the "Drip" formation and the amount of Bound Water during the refrigeration of fish meat.

Section V. The variation of the amount of Bound Water during salting of fish meat.

Section VI. On the relation between the amount of Bound Water and the decomposition of fish meat protein during the autolysis of fish meat.

ACKNOWLEDGEMENT

Before going further, the author wishes to express his hearty thanks to his respected teacher who suggested first this problem, Prof. Eiichi TANIKAWA, Faculty of Fisheries, Hokkaido University, for his sympathetic guidance and encouragement throughout these experiments and his kindness in reading and correcting this manuscript. Moreover, the author wishes to express his obligation to Professors Yukihiko NAKAMURA, and Yataro OBATA, Faculty of Agriculture, Hokkaido University, and to Dr. Masao MIGITA, Tokai Regional Fisheries Research Institute, for their helpful guidance and criticisms in regard to the problems discussed in his paper.

Further, acknowledgements are made to Prof. Ken-ichi HIGASHI, Research Institute of Applied Electricity, Hokkaido University and Prof. Kozo HIROTA, Faculty of Science, Osaka University, for their kind leading and valuable advice.

Lastly, the author wishes to express his thanks to Messrs. Terushige MOTOHIRO, Yutaka FUJII, Yoshio NAGASAWA for their kind aids in these experiments.

II. ESTIMATION OF THE AMOUNT OF BOUND WATER IN FISH MEAT IN VARIOUS CONDITIONS BY THE COBALTOUS CHLORIDE AND BY THE ELECTRICAL RESISTANCE METHODS

In this experiment, the amounts of Bound Water in raw fresh meat, dried meat, salted meat, fermented meat and decomposed meat of various kinds of fish were estimated by the cobaltous chloride method, and by the electrical resistance method.

1. Experimental methods

(1) The method of estimating the amount of Bound water

The method chosen for estimating the amount of Bound Water was Oyagi's[14b)]. The calculation was carried out by the same method as described in the earlier Paper I, III. 1, (II, B)[1a)].

To calculate the amount of Bound Water (R.B, %) in the original samples (not dyed sample), the total amount of water (R.T, %) in the like same sample of each material was determined by the usual drying method.

The calculating formula is as follows:

$$\text{R.B.}=\frac{(100-\text{R.T.})(W_1-W_2)}{W_2} \qquad (1)$$

where, W_1 is the weight at the point where the colour of the dyed sample changes from pink to pure blue, or the apparent constant weight of the sample during the initial drying at 30°C, W_2 is the constant weight of the sample during the final drying at 110°C. The amount of Free Water (R.F. %) in the original sample, is calculated as follows:

$$\text{R.F.}=\text{R.T.}-\text{R.B.} \qquad (2)$$

In this calculation, the amount of Bound Water per gm of the dried matter of the sample was supposed to remain unchanged during the steeping of the sample in 10% cobaltous chloride solution.

(2) The electrical resistance method

The estimating method by the electrical resistance was the same as in the previously described Paper I, III, 1, (1), (IV)[1a]. The sample of fish meat fillet (4×2×2 cm) was impaled upon two copper poles (6.7 cm in length and 0.17 mm in diameter) fixed on the insulated bakelite plate at definite distance. The estimation of the electrical resistance was made by an apparatus manufactured by Fuji Radio Co., its cycle was 50, and its type was wheatstone's bridge. The estimating temperature was 17°C, and the drying temperature of fish meat was 19°C or 20°~25°C; the sample thrust through by the poles was placed in the desiccator before the estimation of electrical resistance.

2. Results and consideration

(1) *Determination of the amount of Bound Water by Oyagi's method*

The results on sandfish (*Arctoscopus japonicus* STEINDACHNER), squid (*Ommastrephes sloani pacificus*) and flat fish (*Kareius bicoloratus* BASILEWSKY) are shown in Table 1 and Fig. 1.

As stated in earlier Paper I, III, 1[1a], the sign B.P. in Table 1 and Fig. 1 is the point at which the colour of pieces of dyed fish meat steeped in the cobaltous chloride solution turns to pure blue from pink in the course of drying at 30°C. The amount of water estimated at point B.P. is represented as the amount of Bound Water. But as shown in the experiment there is another point, C.P., which is recognized as the apparent constant weight of the sample in the course of drying at 30°C from 1 to 3 hours after the appearance of the point B.P.

Discussing Bound Water from its binding strength, any amount of the water content determined by the cobaltous chloride method between the points B.P. and C.P. is regarded as the amount of Bound Water. The author, therefore, calls the amount of water at point B.P. the maximum value of the amount of Bound Water, and that amount at C.P. the minimum value of the amount of Bound Water. The amounts of Bound Water at those two points were estimated on the samples of various kinds of fish.

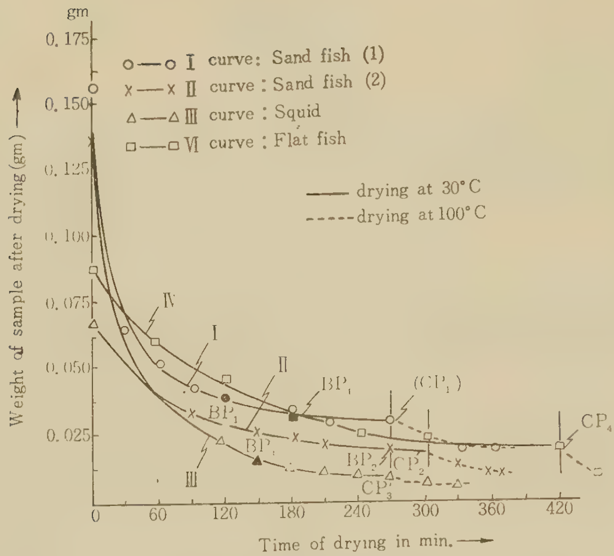
(i) Determination of the amount of Bound Water by the cobaltous chloride method on the raw fresh meat of various kinds of fish

The results derived from using Oyagi's method on the raw fresh meat of

Table 1. Variation in the amounts of water during the drying of various kinds of fish meats by the cobaltous chloride method (Oyagi's method)

Drying time in min.	Sandfish meat			Squid meat			Flat fish meat		
	Change of the weight of sample (gm)	g^* (gm)	Drying temp. (°C)	Change of the weight of sample (gm)	g^* (gm)	Drying temp. (°C)	Change of the weight of sample (gm)	g^* (gm)	Drying temp. (°C)
0	0.1352	10.76	25° ~ 30°C	0.0641	5.04	25° ~ 30°C	0.0883	3.57	15° ~ 20°C
30	0.0534	3.63		—	—		—	—	
60	0.0418	2.63		—	—		0.0613	2.17	
90	0.0319	1.77		—	—		—	—	
120	0.0282	1.39		0.0242	1.28		0.0476	1.46	
150	0.0265	1.30		0.0173	0.63 (B.P_3)		—	—	
180	0.0242	1.10		0.0138	0.30		0.0331	0.72 (B.P_4)	
210	0.0220	0.91 (B.P_2)		0.0130	0.226		—	—	
240	—	—		0.0120	0.132		0.0253	0.31	
270	0.0196	0.70		0.0120	0.132(C.P_3)		—	—	
300	0.0195	0.69 (C.P_2)		—	—	100° ~ 105°C	0.0231	0.198	
330	0.0157	0.36	100° ~ 105°C	0.0106	0.00		—	—	
360	0.0115	0.00		0.0106	0.00		0.0228	0.180(C.P_4)	
370	0.0115	0.00					—	—	
420							0.0228	0.180	
480							0.0198	0.010	100° ~ 105°C
540							0.0193	0.00	
600							0.0193	0.00	
Remarks	II curve in Fig. 1			III curve in Fig. 1			IV curve in Fig. 1		

Note; I curve in Fig. 1 is the same curve as shown in Fig. 7, previous Paper I, Experiment III, 1[1a].
*g=Gm of water per gm of dried matter.



The amount of Bound water per gm of dried matter

0.67 (B.P$_1$)	0.356 (C.P$_1$)
0.91 (B.B$_2$)	0.69 (C.P$_2$)
0.63 (B.P$_3$)	0.132 (C.P$_3$)
0.72 (B.P$_4$)	0.180 (C.P$_4$)
at B.P (max.)	at C.P (min.)

Fig. 1. Variation in the amounts of water during the drying of various kinds of fish meats by the cobaltous chloride method (Oyagi's method)

squids (*Ommastrephes sloani pacificus* and *Doryteuthis bleekeri* KEFERSTEIN), flat fishes (*Microstomus achne* JORDAN *et* STARKS, *Atheresthes evermanni* JORDAN *et* STARKS, *Kareius bicoloratus* BASILEWSKY, *Limanda herzensteini* JORDAN *et* SNYDER and *Paralichthys olivaceus* TEMMINCK *et* SCHLEGEL), herring (*Clupea pallasii*), sardine (*Engraulis japonicus* TEMMINCK *et* SCHLEGEL), mackerel (*Scomber japonicus* HOUTTUYN), Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ), horse mackerel (*Trachurus japonicus* TEMMINCK *et* SCHLEGEL), sand-fish (*Arctoscopus japonicus* STEINDACHNER) and globe-fish (*Spheroides rubripes* TEMMINCK *et* SCHLEGEL) are shown in Tables 2, 3, and 4. Table 2 shows the minimum values of the amount of Bound Water in the samples; Table 3 shows the maximum values of Bound Water; Table 4 shows the averages of those values of Bound Water per 100 gm of the dried matter of the samples.

From Table 4 it is clear that the minimum amount of Bound Water is 10~48 gm (24.4 gm on the average) per 100 gm of the dried matter, and the maximum amount of Bound Water is 55~82 gm (63.2 gm on the average). Supposing that Free Water has evaporated first when the raw fresh meat is dried, therefore, the total amount of water remaining in the fish meat is considered to be Bound Water at the time when the water-content of the meat shows

Table 2. Minimum values of the amount of Bound Water in the raw fresh meat of various kinds of fish

Samples \ Water-contents		Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.F.}{R.T.} \times 100$ (%)	$\frac{R.B.}{R.T.} \times 100$ (%)	"g" (gm of Bound Water per gm of dried matter)	$\frac{g}{g+D.M.} \times 100$ (%) (D.M.=Dried matter)
Squid (*Ommastrephes sloani pacificus*)	(1)	83.51	81.43	2.18	97.50	2.50	0.132	11.6
	(2)	81.64	80.64	3.48	95.85	4.15	0.220	18.0
Squid (*Doryteuthis bleekeri* KEFERSTEIN)		79.89	70.26	9.60	88.00	12.00	0.477	32.3
Flat fish (*Atheresthes evermanni* JORDAN *et* STARKS)		80.99	79.28	1.71	97.87	2.13	0.09	8.25
Flat fish (*Kareius bicoloratus* BASILEWSKY)	(1)	79.19	74.51	4.66	94.11	5.89	0.224	18.3
	(2)	78.64	74.39	4.25	94.59	5.41	0.169	16.6
Flat fish (*Paralichthys olivaceus* TEMMINCK *et* SCHLEGEL)		78.02	73.30	4.72	93.90	6.10	0.215	17.7
Flat fish (*Microstomus achne* JORDAN *et* STARKS)		76.18	72.50	3.68	95.16	4.84	0.154	13.3
Herring (*Clupea pallasii*)	(1)	81.64	78.84	2.80	96.50	3.44	0.152	13.1
	(2)	80.62	76.70	3.92	95.13	4.87	0.202	16.6
Sardine (*Engraulis japonicus* TEMMINCK *et* SCHLEGEL)	(1)	76.55	71.80	4.37	93.80	6.20	0.202	16.6
	(2)	79.34	74.54	4.80	94.00	6.00	0.232	18.8
Sandfish (*Arctoscopus japonicus* STEINDACHNER)		80.30	73.30	7.00	91.28	8.72	0.356	26.2
Atka mackerel (*Preurogrammus azonus* JORDAN *et* METZ)	(1)	80.00	71.26	8.74	89.09	10.93	0.437	30.4
	(2)	83.95	80.47	3.48	95.85	4.15	0.216	17.8
	(3)	80.99	79.28	1.71	97.87	2.13	0.090	8.25

Table 3. Maximum values of the amount of Bound Water in the raw fresh meat of various kinds of fish

Samples \ Water-contents	Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.F.}{R.T.} \times 100$ (%)	$\frac{R.B.}{R.T.} \times 100$ (%)	"g" (gm of Bound Water per gm of dried matter)	$\frac{g}{g+D.M.} \times 100$ (%) (D.M. = Dried matter)
Squid (*Doryteuthis bleekeri* KEFERSTEIN)	(1) 76.27 (2) 78.19 (3) 78.73 (4) 78.45	59.03 60.85 59.56 60.21	17.24 17.34 19.17 18.24	77.3 77.8 75.6 76.7	22.7 22.2 24.4 23.3	0.726 0.778 0.896 0.845	42.0 43.8 47.2 45.8
Squid (*Ommastrephes sloani pacificus*)	76.62	63.58	13.58	82.9	17.1	0.557	35.8
Sandine (*Engraulis japonicus* TEMMINCK et SCHLEGEL)	(1) 76.70 (2) 76.70	65.66 59.50	11.04 17.20	85.6 77.5	14.4 22.5	0.474 0.738	32.1 42.5
Sandfish (*Arctoscopus japonicus* STEINDACHNER)	(1) 80.51 (2) 80.51	67.40 66.79	13.11 13.72	83.7 82.9	16.3 17.1	0.678 0.704	40.2 41.3
Horse mackerel (*Trachurus japonicus* TEMMINCK et SCHLEGEL)	(1) 76.14 (2) 73.20	57.60 54.28	18.54 18.92	75.7 74.1	24.3 25.9	0.777 0.706	43.7 41.4
Grobe-fish (*Spheroides rubripes* TEMMINCK et SCHLEGEL)	(1) 80.07 (2) 80.10	68.97 65.40	11.10 14.67	86.1 81.0	13.9 19.0	0.557 0.736	35.8 42.4
Flat fish (*Limanda herzensteini* JORDAN et SNYDER)	(1) 78.88 (2) 78.74	52.96 54.99	25.92 23.75	67.1 69.8	32.9 30.2	1.230 1.117	55.2 52.8
Mackerel (*Scomber japonicus* HOUTTUYN)	76.62	63.58	13.04	82.9	17.1	0.557	35.8

Table 4. Amount of Bound Water in the raw fresh meat of various kinds of fish

Samples \ Amount of Bound Water	Minimum amount of Bound Water*	Maximum amount of Bound Water*
Squid (*Ommastrephes sloani pacificus*)	17.5	55.7
Squid (*Doryteuthis bleekeri* KEFERSTEIN)	47.7	81.1
Sardine (*Engraulis japonicus* TEMMINCK *et* SCHLEGEL)	21.7	60.6
Sandfish (*Arctoscopus japonicus* STEINDACHNER)	35.6	68.8
Flat fish (*Kareius bicoloratus* BASILEWSKY)	21.1	—
Flat fish (*Microstomus achne* JORDAN *et* STARKS)	9.0	—
Flat fish (*Atheresthes evermanni* JORDAN *et* STARKS)	15.4	—
Flat fish (*Paralichthys olivaceus* TEMMINCK *et* SCHLEGEL)	21.5	—
Flat fish (*Limanda herzensteini* JORDAN *et* SNYDER)	—	54
Herring (*Clupea pallasii*)	17.5	—
Atka mackerel *Pleurogrammus azonus* JORDAN *et* METZ)	37.1	—
Mackerel (*Scomber japonicus* HOUTTUYN)	—	55.7
Horse mackerel (*Trachurus japonicus* TEMMINCK *et* SCHLEGEL)	—	64.8
Globe-fish (*Spheroides rubripes* TEMMINCK *et* SCHLEGEL)	—	64.6
Range (Average)	10~48 (24.4)	55~82 (63.2)

* gm of Bound Water per 100 gm of dried matter.

about 20% (the minimum water-content) or 40% (the maximum water-content) in original material.

Higuchi[12] has estimated the amounts of Bound Water in squid and flat fish meat by a method concerning the depression of the freezing point and calorimetric method. The present author has calculated Higuchi's results as follows: the amount of Bound Water in squid is 13.4 gm per 100 gm of the dried matter of the sample, and 18.7 gm for flat fish meat. These values are in correspondence with the minimum values estimated by this cobaltous chloride method.

(ii) The amount of Bound Water estimated in the dried meat of several kinds of fish by the cobaltous chloride method

The maximum and minimum values of the amount of Bound Water of squids (*Ommastrephes sloani pacificus* and *Doryteuthis bleekeri* KEFERSTEIN), mackerel (*Scomber japonicus* HOUTTUYN), Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ), sandfish (*Arctoscopus japonicus* STEINDACHNER), globefish (*Spheroides rubripes* TEMMINCK *et* SCHLEGEL), sardine (*Engraulis japonicus* TEMMINCK *et* SCHLEGEL) and flat fish (*Limanda herzensteini* JORDAN *et* SNYDER) by the cobaltous chloride method are shown in Tables 5 and 6.

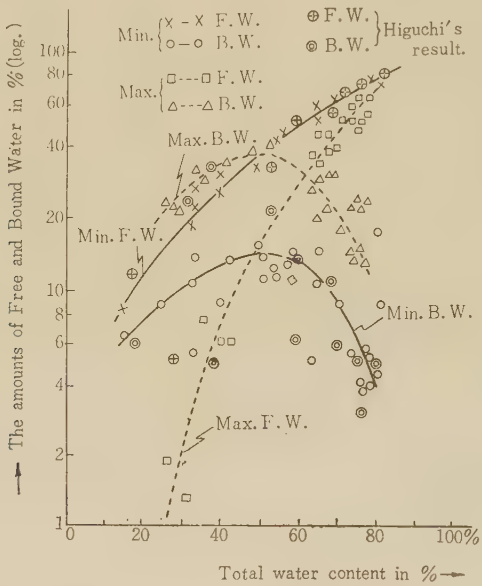


Fig. 2. Variation of the amounts of Free (F.W.) and Bound Water (B.W.) in the course of drying

Fig. 2 shows the relation between the total amount of water of each sample of various kinds of fish meat and the amount of Free Water, or the relation between the maximum and the minimum values of Bound Water of each sample.

Fig. 3 shows the amount of Bound Water per gm of the dried matter of each sample in relation to the total amount of water in each sample. The curves in Fig. 2 are formed by joining the massed points for the amounts of Free Water and Bound Water (the maximum and the minimum values) of all the samples. These curves are considered to show the variation of the amounts of Free and Bound Water during the drying of fish meat.

From Fig. 2 it is clear that with the drying, the percentage of the amount of Free Water decreases, while that of Bound Water increases relatively; the relative amount of Bound Water to the total amount of water shows the maximum at about 40~50% of the total amount of water in the fish meat. At this peak of Bound Water content, the percentage of the amount of Bound Water to the total

Table 5. Minimum values of the amount of Bound Water in the dried meat of various kinds of fish

Samples and Treatments \ Water-contents		Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.F.}{R.T.} \times 100$ (%)	$\frac{R.B.}{R.T.} \times 100$ (%)	"g" (gm of Bound Water per gm of dried matter)	$\frac{g}{g+D.M.} \times 100$ (%) (D.M.=Dried matter)
Squid (*Ommastrephes sloani pacificus*) (Sun-drying)	(1)	77.33	73.33	4.00	94.80	5.20	0.176	15.0
	(2)	74.64	69.11	5.53	92.59	7.41	0.218	17.9
	(3)	62.87	57.83	5.04	91.98	8.02	0.136	12.0
	(4)	38.53	26.48	12.05	68.72	31.28	0.196	16.4
	(5)	31.80	21.28	10.52	66.91	33.09	0.154	13.3
	(6)	31.74	18.10	13.64	57.02	42.98	0.199	16.6
	(7)	31.44	26.10	5.34	83.01	16.99	0.078	7.25
	(8)	14.58	8.38	6.20	57.47	42.53	0.073	6.82
Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ) (Semi-dried)	(1)	79.17	74.51	4.66	94.11	5.89	0.224	18.3
	(2)	78.64	74.39	4.25	94.59	5.41	0.199	16.6
	(3)	77.51	69.50	5.01	86.67	10.33	0.356	27.0
	(4)	77.33	73.33	4.00	94.82	5.18	0.176	15.0
	(5)	76.18	72.50	3.68	95.16	4.84	0.154	13.3
	(6)	74.64	69.11	5.54	92.56	7.41	0.218	17.9
	(7)	69.00	60.29	8.71	87.37	12.63	0.281	21.9
Ditto (Sun-drying)	(1)	80.00	71.26	8.74	89.09	10.93	0.437	30.4
	(2)	64.90	54.16	10.74	83.45	16.55	0.306	23.4
	(3)	57.90	46.92	10.98	81.03	18.97	0.261	19.9
	(4)	55.90	43.47	12.45	77.76	22.24	0.282	21.9
Ditto (Drying by heating)	(1)	63.20	48.74	14.46	77.12	22.88	0.393	28.2
	(2)	40.50	27.35	13.15	67.53	32.47	0.221	18.1
	(3)	38.30	29.54	8.76	77.12	22.88	0.142	12.4
Ditto (Salting and Drying)	(1)	57.00	42.43	14.44	74.44	25.56	0.339	25.3
	(2)	55.00	41.64	13.33	72.33	27.67	0.269	21.2
	(3)	52.90	40.80	12.10	77.12	22.88	0.257	20.4
	(4)	49.30	35.66	13.64	72.33	27.67	0.269	21.2
	(5)	48.60	37.65	10.95	74.73	25.27	0.213	17.5
	(6)	47.60	32.99	15.51	67.41	32.59	0.296	22.9

Table 6. Maximum values of the amount of Bound Water in the dried meat of various kinds of fish

Samples and Treatment \ Water-contents		Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.F.}{R.T.}\times100$ (%)	$\frac{R.B.}{R.T.}\times100$ (%)	"g" (gm of Bound Water per gm of dried matter)	$\frac{g}{g+D.M.}\times100$ (%) (D.M.=Dried matter)
Squid (*Doryteuthis bleekeri* KEFERSTEIN) (Sun-drying)	(1)	34.31	7.31	26.93	21.50	78.50	0.410	29.0
	(2)	26.42	1.84	24.58	6.96	93.04	0.334	25.0
	(3)	25.61	0.76	28.85	2.97	97.03	0.334	25.0
Mackerel (*Scomber japonicus* HOUTTUYN) (Semi-dried)	(1)	73.27	50.68	22.59	68.20	30.80	0.845	45.7
	(2)	73.38	50.21	23.17	68.40	31.60	0.873	46.6
	(3)	73.49	49.74	23.75	67.70	32.30	0.896	47.2
	(4)	64.95	43.75	21.20	67.30	32.70	0.605	37.8
Sandfish (*Arctoscopus japonicus* STEINDACHNER) (Sun-drying)	(1)	52.71	11.76	40.95	22.30	77.30	0.862	46.6
	(2)	28.57	5.15	23.42	18.02	81.98	0.328	24.7
Globefish (*Spheroides rubripes*) TEMMINCK *et* SCHLEGEL) (Sun-drying)	(1)	39.47	5.82	33.65	14.70	85.30	0.556	35.7
	(2)	39.47	5.94	33.53	15.00	85.00	0.554	35.6
Squid (*Ommastrephes sloani pacificus*) (Semi-dried)	(1)	74.10	60.17	13.93	81.20	18.80	0.538	35.0
	(2)	73.50	58.93	14.57	80.10	19.90	0.550	35.4
	(3)	73.80	59.55	14.25	80.70	19.30	0.544	35.2
	(4)	70.10	51.26	18.85	73.10	26.90	0.603	37.6
	(5)	63.26	35.38	27.88	55.90	44.10	0.756	43.1
	(6)	66.68	43.99	22.64	65.90	34.10	0.681	40.2
Flat fish (*Limanda herzensteini* JORDAN *et* SNYDER) (Semi-dried)	(1)	76.61	53.06	23.55	69.20	30.80	1.007	50.3
	(2)	68.55	37.98	30.57	55.40	44.60	0.972	49.3
	(3)	65.21	24.98	30.23	53.64	46.34	0.864	46.3
	(4)	66.88	36.80	30.50	54.39	45.61	0.921	47.9
Sardine (*Engraulis japonicus* TEMMINCK *et* SCHLEGEL) (Sun-drying)		31.76	1.26	30.50	3.96	96.04	0.447	30.9

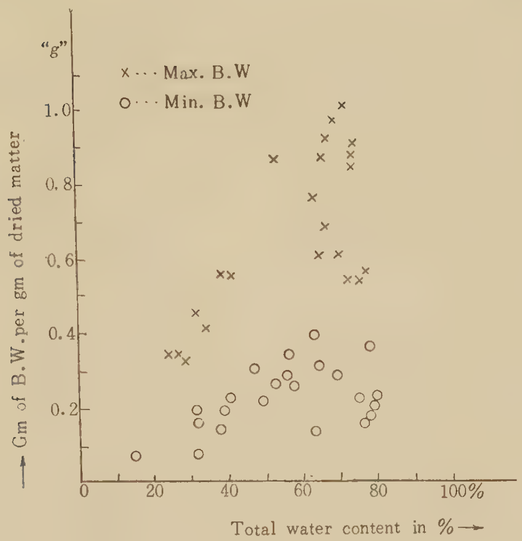


Fig. 3. Distribution of the absolute amount of Bound Water in the course of drying

amount of water in the fish meat shows from about 25% (minimum) to 75% (maximum) as seen in Tables 5 and 6. With further dehydration, the percentage of the amount of Bound Water decreases.

Higuchi's results for squid and flat fish meat during the drying which were together shown in Fig. 2 agreed generally with the minimum value of the amount of Bound Water obtained by the present author. It is interesting that the values of the amount of Bound Water obtained by the thermodynamic methods such as the one concerning the depression of the freezing point and the calorimetric method by Higuchi agreed almost with the results obtained by the chemical methods such as the cobaltous chloride method which is different from the thermodynamic methods in the idea of the determination. This agreement encourages the present author to believe that the minimum value of the amount of Bound Water obtained by the cobaltous chloride method (the amount of water at the point of C.P. in the course of drying of the sample) should be considered as the colloidal Bound Water. This conclusion has also been substantiated by the previously reported Experiment III, 1[1a].

From Fig. 3 the absolute amount of Bound Water per gm of the dried matter of the fish meat apparently decreases generally with the drying of the sample.

(iii) Results obtained by the cobaltous chloride method on the salted fish meat

Tables 7 and 8 show the amounts of Bound Water in the salted fish meat—Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ), squid (*Doryteuthis bleekeri* KEFERSTEIN) and mackerel (*Scomber japonicus* HOUTTUYN).

Salted Atka mackerel meat was prepared by the dry-salting and brine-salting processes respectively. Salted mackerel and squid were made by dry-salting

Table 7. Variation of the minimum values of the amount of Bound Water in Atka mackerel meat at certain intervals after salting

Samples, Treatments and Days	Water-contents	Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.B.}{R.T.}\times 100$ (%)	$\frac{R.F.}{R.T.}\times 100$ (%)	"*g*" (gm of Bound Water per gm of dried matter)
Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ) (Brine-salting)	Raw fish meat	80.5	68.92	11.58	14.39	85.61	0.594
	3 days after	61.7	44.73	16.97	27.53	72.47	0.443
	5 〃	62.8	50.52	12.28	19.56	80.44	0.330
	7 〃	63.1	—	—	—	—	—
Ditto (Dry-salting)	Raw fish meat	80.5	68.92	11.58	14.39	85.61	0.594
	3 days after	61.1	47.18	13.92	22.88	77.12	0.358
	5 〃	59.7	42.62	17.08	28.60	71.40	0.425
	7 〃	42.4	20.80	21.60	59.95	49.50	0.375

Table 8. Variation of the maximum values of Bound Water in squid and mackerel meat at certain intervals after salting

Samples, Treatments and Days	Water-contents	Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.B.}{R.T.}\times 100$ (%)	$\frac{R.F.}{R.T.}\times 100$ (%)	"*g*" (gm of Bound Water per gm of dried matter)
Squid (*Doryteuthis bleekeri* KEFERSTEIN) (Dry-salting)	Raw fish meat	78.45	60.21	18.24	23.26	76.74	0.845
	1 day after	62.52	33.73	29.79	46.05	53.95	0.795
	2 days after	61.94	14.41	47.53	76.74	23.26	1.249
	4 〃	60.44	13.24	47.20	78.10	21.90	1.193
Mackerel (*Scomber japonicus* HOUTTUYN) (Dry-salting)	Raw fish meat	73.38	50.21	23.17	31.58	68.42	0.873
	1 day after	58.04	9.62	48.42	83.43	16.57	1.154
	2 days after	55.65	10.32	45.33	81.46	18.54	1.022
	4 〃	57.94	26.68	31.30	53.99	46.01	0.745

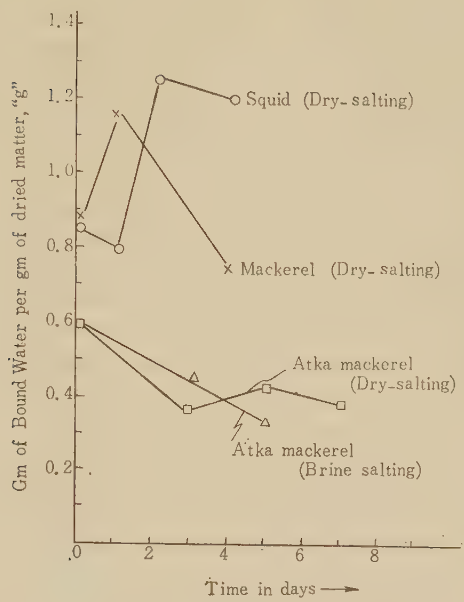


Fig. 4. Variation of the absolute amount of Bound Water in the course of salting

process. The amount of NaCl used was 20% by weight of the raw material in the dry-salting process. The brine used was saturated NaCl solution. The amounts of Bound Water in these salted fishes were determined by the cobaltous chloride method (Oyagi's).

Fig. 4 shows the variation of the absolute amounts of Bound Water in the salted Atka mackerel, squid and mackerel meat at certain intervals after salting.

In Table 7 the minimum values of Bound Water (at C.P.) for Atka mackerel meat were tabulated, while in Table 8 the maximum values of Bound Water (at B.P.) for mackerel and squid meat were tabulated.

From Tables 7 and 8, it is observed that the total amount of water decreases with the lapse of days after the salting process because of the osmotic dehydration by salt occurs. The dehydrating action of the dry-salting is greater than that of the brine-salting. This fact agrees with the previous results of many investigators[24].

During the salting of fish meat, the percentage of the amount of Free Water decreases, while that of Bound Water relatively increases; this fact agrees with the case of the dried fish meat.

However, the absolute amount of Bound Water in the Atka mackerel meat per gm of the dried matter decreased somewhat with the lapse of days after the salting, as shown in Fig. 4. This fact is perhaps due to the dissolution of fish meat protein or to the denaturation of the protein by the salting.

In the case of squid and mackerel meat the decrease in the absolute amount of Bound Water is not so clear as observed in Atka mackerel meat as shown in Fig. 4.

(iv) Results of estimations of the amount of Bound Water in fermented fish meat and decomposed fish meat by the cobaltous chloride method

(A) Variation of the amount of Bound Water in soused-squid meat ("Shiokara" in Japanese) during its ripening

The variation of the amount of Bound Water in soused-squid meat was observed as follows:

Soused-squid meat ("Shiokara") is a Japanese special product which is made from cut squid meat, its liver, and salt (sodium chloride). The author has prepared it by the following process. The body of fresh squid (average weight of a squid is 284 gm) was cut in small rectangular pieces (0.5 × 3 cm), and was mixed with NaCl and squid liver, then was stirred every day for 4 weeks. The amount of NaCl added was 15% by weight of the cut squid meat. The amount of squid liver added was 3% (A), and 6% (B), respectively by weight, of the cut squid meat.

The amount of Bound Water in the soused-squid meat was estimated by the cobaltous chloride method (Oyagi's method) after washing the surface of the cut squid meat and absorbing the water attached to the surface of the meat.

The variation of the amount of Bound Water during the ripening of soused-squid ("Shiokara") is shown as (A) and (B) in Table 9. The values there tabulated are the minimum amounts of Bound Water (at C.P.).

From Table 9 (A), (B), it is observed that the total amount of water increased gradually with the decomposition of fish meat protein by enzymic action for the first 10 days, and then that amount decreased as a manifestation of the effect of dehydrating action. In the total amount of water, the amount of Free

Table 9. Variation of the minimum amount of Bound Water during the ripening of soused-squid meat (" Shiokara ")

Days of processing	Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.F.}{R.T.}\times 100$ (%)	$\frac{R.B.}{R.T.}\times 100$ (%)	"g" (gm of Bound Water per gm of dried matter)
(A) Squid liver added in 3%						
0	82.63	79.38	3.25	96.06	3.94	0.187
4	85.66	82.95	2.71	96.83	3.14	0.189
7	89.00	97.38	1.62	98.17	1.83	0.147
10	82.44	82.01	0.43	99.47	0.53	0.024
21	87.46	87.22	0.24	99.72	0.28	0.019
(B) Squid liver added in 6%						
0	82.63	79.38	3.25	96.06	3.94	0.187
4	88.54	85.84	2.70	96.95	3.05	0.232
7	89.03	86.63	2.40	97.30	2.70	0.219
10	81.15	80.09	1.06	98.69	1.31	0.056
21	82.17	82.20	0.97	98.81	1.19	0.054

Water increased gradually and that of Bound Water decreased. This fact is contrary to the results obtained with the dried fish meat or the salted fish meat. The absolute amount of Bound Water per gm of the dried matter of the fermented fish meat decreased during the ripening. But this amount was observed to increase contrarily during the incipient ripening of fish meat. This fact will be understood that the dried matter of fish meat decreases owing to the decomposition of fish meat protein, but the amount of Bound Water is hardly influenced. However, this consideration is somewhat doubtful because of the supposition that the amount of Bound Water did not vary during the steeping of the sample in cobaltous chloride solution. It is certain that the variation of the amount of Bound Water in the fermented squid meat is different from that in either the dried fish meat or the salted fish meat.

(B) Variation of the amount of Bound Water in the decomposed Atka mackerel meat during the putrefaction

Semi-dried Atka mackerel meat (total water-content, 69%) was left to the time of detecting tainted odour in the Petri dish at 30°C, and the variation of the amount of Bound Water during the putrefaction was observed. The experimental results are shown in Table 10.

Table 10. Variation of the minimum amount of Bound Water in the decomposed Atka mackerel meat during the putrefaction

Days of processing	Total Water (R.T. %)	Free Water (R.F. %)	Bound Water (R.B. %)	$\frac{R.F.}{R.T.}\times 100$ (%)	$\frac{R.B.}{R.T.}\times 100$ (%)	"g" (gm of Bound Water per gm of dried matter)
0	69.0	60.29	8.71	87.37	12.63	0.281
2	69.4	62.24	7.16	89.68	10.32	0.237
6	74.2	67.39	6.81	90.82	9.18	0.264

As shown in Table 10, when the muscle tissue of fish decomposed with the putrefaction, the total amount of water increased gradually, and the tainted odour was noticeable already a day after the handling. It was observed that the amount of Free Water increased, while that of Bound Water decreased with the putrefaction of fish meat. This fact is similar to that obtained in the fermented squid meat. However, the variation of the absolute amount of Bound Water per gm of the dried matter of the sample is not clear.

In this experiment employing the cobaltous chloride method, the estimation was very difficult owing to the softening of fish meat; estimation was made again by the electrical resistance measurement. The vapour tension method was also applied for estimating the amount of Bound Water in the putrefied fish meat, but this method was not satisfactory, because the volatile basic matters grew from the meat.

(2) *Results of estimations by the electrical resistance method on some fish meat*

Ueda, Ito and Nishi[25a)] and Tamura[25b)] have observed the variation of the

electrical resistance in the fresh carp meat after the death. They stated that the electrical resistance decreased rapidly at a definite time after the death, and the lower the temperature is, the longer the time needed to decrease electrical resistance. The cause of the decreasing of electrical resistance is perhaps due to the change of the muscle tissue of fish meat by autolysis and bacterial action. Yamamura[25c)] has observed that the specific electrical resistance of the direct current is 2,400～3,000 Ohms and of the alternating current of 1,000 cycles is 80～90 Ohms for the raw fresh meat of horse mackerel (*Trachurus japonicus* TEMMINCK *et* SCHLEGEL), mackerel (*Scomber japonicus* HOUTTUYN) or flat fish (*Limanda herzensteini* JORDAN *et* SNYDER). Tamura, Miyazaki and Kaziyama[25d)] have studied the relation between the penetrated amount of NaCl into fish meat and the electrical resistance in the salted fish meat. Callow[25e)] has also studied the electrical resistance of the muscular tissue of farm-killed pigs and of pigs killed in factory or abattoir. Recently Yamada and Kitano[25f)] have studied variation of the electrical resistance and of pH value of the denatured myosin added with NaCl. Ito, Kyozuka and Sugizaki[25g)] have also studied the electrical resistance of raw fresh fish meat, heated fish meat and chilled fish meat.

The present author has studied in his earlier Paper I, Experiment III, 1[1a)], the variation of the electrical resistance at 50 cycles in sample of gelatine and fish meat having various water-contents, and has obtained following results: the electrical resistance decreased somewhat with the initial decreasing of water-content, but it increased gradually at point above a definite dryness, and thereafter it increased rapidly. The gradual increasing of the electrical resistance at above a definite dryness was considered to be due to the increasing of the amount of Bound Water in inverse proportion to the decreasing of the Free Water. The rapid increasing in the next stage was considered to be due to the increasing of the binding strength of the Bound Water remained.

The amount of water estimated at point of rapid increasing of the electrical resistance was considered to be colloidal Bound Water as compared with the results obtained by the cobaltous chloride and vapour tension methods.

Here, the author reports the results obtained by estimating the electrical resistance for some fish meat.

(i) Preliminary experiment

(A) Relation between the electrical resistance (50 cycles) and the distance of electrodes for various kinds of fish meat

Table 11 and Fig. 5 show the relation between the electrical resistance at 50 cycles and the distance of the electrodes for various kinds of fish meat. In Fig. 5, the results obtained by Yamamura[25c)] are written together. It was observed that the electrical resistance was proportional to the distance of electrodes and showed liner function. This result agrees with that obtained by Yamamura, but it was contrary to the result of Ito, Kyozuka and Sugizaki[25g)].

The electrical resistance of unfresh raw or chilled Atka mackerel meat is less than that of fresh raw Atka mackerel meat. This result agrees with the results obtained by Tamura[25b)]. In salted Atka mackerel meat, the electrical resistance decreased with the penetration of the electrolyte (NaCl).

Table 11. Relation between the electrical resistance at 50 cycles and the distance of the electrodes for various kinds of fish meat

Samples / Dist. of electrodes	Whole body of a species of Surf-smelt	Fesh raw Atka mackerel meat	Chilled Atka mackerel meat	Salted Atka mackerel meat	Unfresh raw Atka mackerel meat	Fresh raw squid (*Doryteuthis bleekeri*) meat
1.5 cm.	0.395 $K\Omega$	0.266	—	—	0.162	0.77
2.0	0.400	—	—	0.030	0.178	1.11
2.5	0.530	0.363	—	—	—	1.25
3.0	—	—	0.063	0.040	0.194	—
3.5	—	—	—	0.048	0.228	1.45
4.0	—	0.455	—	—	—	—
4.7	—	—	—	—	0.268	2.00
5.0	0.655	0.540	—	0.050	—	—
5.4	—	—	0.075	—	0.298	—
6.0	0.780	0.576	—	0.060	0.305	—
6.9	—	0.700	—	—	0.332	—
8.0	—	0.735	0.083	—	0.378	—
9.0	—	0.870	—	—	0.453	—
10.0	—	0.950	0.105	—	0.485	—
Remarks	Water-content 75.8%			Water-content 70.2%, NaCl 15.7%		

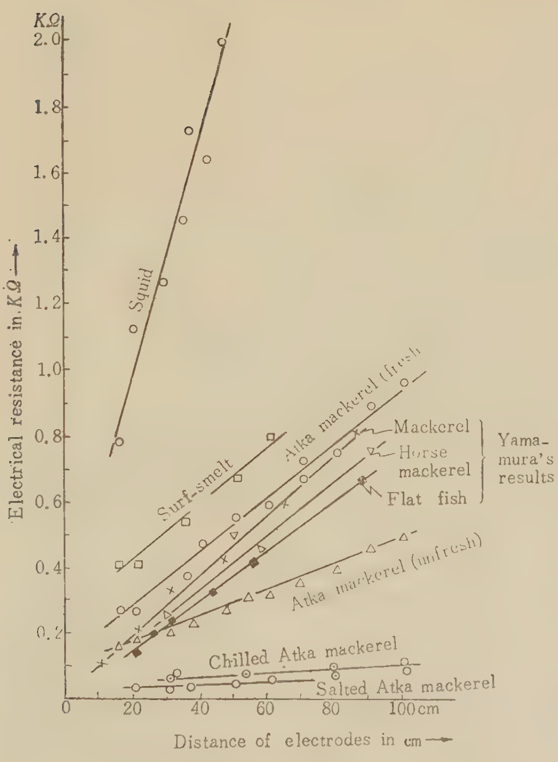


Fig. 5. Relation between the electrical resistance at 50 cycles and the distance of electrodes for various kinds of fish meat (Temp., 15°~16°C)

(B) The electrical resistance of fish meat gruel

After the method employed by Tamura[25b)], the present author ground 100 gm of raw fresh meat of Atka mackerel and filtered it through gauze. This filtered meat was added with 200 c.c. of distilled water and made into fish meat gruel. It was separated in two parts: (A) Antiseptized gruel with toluene, (B) Non-antiseptized gruel. The electrical resistance of these two samples was estimated in a glass desiccator for several days.

The electrodes used were two platinum black plates 2 × 2 cm in size. They were fixed keeping them at a distance of 3 cm from each other in fish meat gruel. The specific electrical resistance was not estimated, but the apparent electrical resistance was estimated. The results are as shown in Table 12.

Table 12. Variation in the electrical resistance of fish meat gruel (Atka mackerel meat) at certain intervals

Processing \ Time (hrs.)	0	15	21	63	87	111	135	159	183	231
(A) Antiseptized gruel by toluene	160	160	155	185	180	170	170	175	170	170
(B) Non-antiseptized gruel	160	160	150	160	150	150	150	145	150	150
Remarks	pH {A. 6.3, B. 6.6	6.3, 6.6	—, 6.4							pH {A. 6.4, B. 6.8

The electrical resistance of two samples (A) and (B) almost did not vary with the lapse of time after the handling. This result agrees with the results obtained by Tamura[25b)].

From the result obtained, it was considered that the depression of the electrical resistance observed in the unfresh fish meat in previous section (A) is not due to the increasing of the amount of the soluble matter by the autolysis of fish meat or the decomposition of meat protein by bacteria, but that it is due to the destruction of muscle tissue of the meat.

(C) The relation among the water-content in the putrefied fish meat, the electrical resistance and the distance of the electrodes

From the previous two experiments, it was observed that the electrical resistance decreased with the destruction of the muscle tissue of fish meat.

Here, the present author has undertaken to study the relation among the water-content in the fish meat putrefied in the incipient drying, the electrical resistance and the distances of the electrodes.

The samples used were fresh raw Atka mackerel meat fillet (11×1.5×1.0 cm) and the whole body of a species of surf-smelt (*Hypomesus japonicus*), (10 gm in weight, 13 cm in length).

The distance between the electrodes was 1.5 to 10 cm.. The electrical resistances were observed for the samples two times in the incipient drying, and were observed thereafter for the putrefied samples which were laid in the Petri dishes at 30°C and began to have tainted smell. The putrefaction of fish meat

was determined by Amano's $HgCl_2$[26] reaction. The electrical resistance of those samples was estimated at various water-content during the air-drying. The estimation of the electrical resistance for the whole body of surf-smelt was carried out as follows: two electrodes were thrust through into the center part of fish body between the body surface and back bone at the lateral line, and the electrical resistance was estimated at various distances of electodes.

The experimental results are shown in Tables 13 and 14. The average value of three samples is tabulated. The relation between the electrical resistance and the distance of electrodes is illustrated in Fig. 6, and the relation among the

Table 13. Relation among the water-contents in the putrefied Atka mackerel meat, the electrical resistance (50 cycles) and the distance of electrodes

Water-content (%) \ Distance of electrodes (cm)	1.5	2.0	3.0	3.7	4.7	5.4
	KΩ					
75.0	0.163	0.178	0.194	0.228	0.268	0.298
72.6	0.176	0.167	0.218	0.258	0.295	0.331
70.0	0.208	0.238	0.256	0.296	0.325	0.371
60.4	0.141	0.154	0.234	0.236	0.285	0.344
50.3	0.141	0.217	0.345	0.410	0.479	0.563

Water-content (%) \ Distance of electrodes (cm)	6.1	6.9	8.0	9.0	10.0	Remarks
75.0	0.305	0.330	0.378	0.453	0.485	Drying
72.6	0.363	0.376	0.416	0.448	0.495	//
70.0	0.402	0.427	0.498	0.546	0.605	//
60.4	0.366	0.442	0.507	—	0.757	(Putrefied)
50.3	0.656	0.503	0.979	1.393	1.550	Drying

Table 14. Relation among the water-contents in the putrefied meat of a species of surf-smelt (whole body), the electrical resistance (50 cycles) and the distance of electrodes

Water-content (%) \ Distance of electrodes (cm)	1.5	2.0	3.5	5.0	6.0	Remarks
	KΩ					
75.4	0.390	0.380	0.535	0.663	0.813	Drying
72.5	0.390	0.415	0.550	0.738	0.825	//
69.4	0.430	0.502	0.728	0.988	1.240	//
64.7	0.336	0.393	0.638	0.913	1.192	(Putrefied)
55.7	0.421	0.457	0.866	1.373	2.085	Drying
40.3	0.640	0.817	1.618	5.325	30.533	//
25.0	1.708	2.255	43.25	778	8,000 over	//

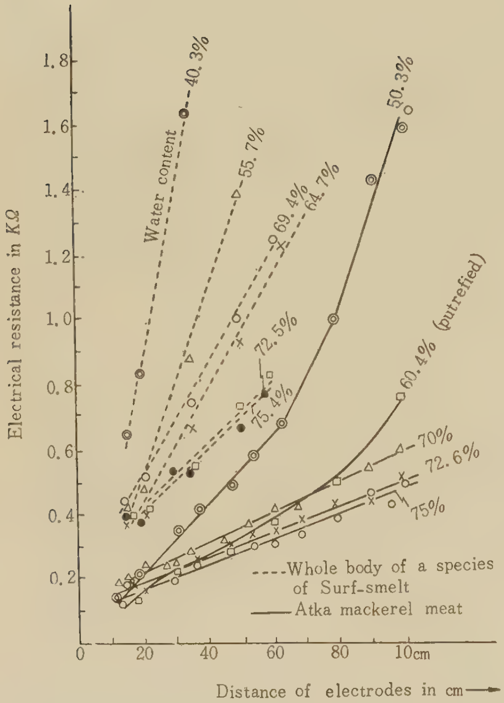


Fig. 6. Relation among the electrical resistance (50 cycles), the distance of the electrodes and the total water-contents in fish meat in various conditions



Fig. 7. Relation among the water contents in the putrefied fish meat, the electrical resistance and the distance of the electrodes

electrical resistance, the water-content and the distance of electrodes is illustrated in Fig. 7.

From Table 13 and Fig. 6, it was observed that the relation between the electrical resistance of Atka mackerel meat and the distance of the electrodes was a linear function, and with the drying of meat the straight line slipped off upwards. But the electrical resistance of the completely putrefied Atka mackerel meat (water-content 60.4%) depressed temporarily with the decreasing of water content and no linear function was observed, as if this result was inconsistent to the raw fish meat as above described. This fact is perhaps due to the destruction of the muscle tissue of the fish meat. But the electrical resistance increased with the progressive drying of the putrefied fish meat. Then the straight line showing the relation between the electrical resistance and the distance of electrodes slipped off upwards. At excessive drying, the electrical resistance increased logarithmically as indicated in Fig. 7.

As observed in Atka mackerel meat, the straight line showing the relation

between the electrical resistance of surf-smelt and distance of electrodes slipped off upwards with the drying. The electrical resistance of the putrefied fish meat also decreased temporarily with the decreasing of the water-content, then it increased logarithmically with the drying.

The electrical resistance of dried fish meat without putrefaction could be estimated. However, supposing from Figs. 6 and 7 showing the comparison of the curves of electrical resistance at the beginning of drying with after the putrefaction, the curve of the electrical resistance of the fish meat dried from fresh raw meat without putrefaction, perhaps slips off left side from the curve of the electrical resistance of the putrefied meat. As stated in earlier Paper Experiment III, 1[1a], if the rapid ascending of the curve of the electrical resistance in the lower water-content is due to the existence of the Bound Water having a certain degree of binding strength with fish meat protein, the fact that the rapid ascending point of the curve obtained from the putrefied fish meat was indicated in the lower water-content than in the fresh raw fish meat is perhaps due to the decreasing of the amount of Bound Water or to the weakening of the binding strength of it owing to the destruction of muscle tissue of fish meat.

(ii) Results of observations of the electrical resistance at 50 cycles in fresh meat, salted meat and putrefied meat of Atka mackerel at various water-contents

In this experiment, use was made of fresh raw Atka mackerel meat ($8 \times 1.8 \times 1.0$ cm), putrefied Atka mackerel which has a slight putrefied smell in incipient drying and in the same size, and brine-salted Atka mackerel meat which has been immersed in saturated NaCl solution for 5 hours. The distance between the electrodes was 1.5 cm. The observed results of the electrical resistance at 50 cycles at various water-contents are shown in Tables 15 and 16. The results on the amount of Bound Water for the same samples by the cobaltous chloride method are also shown in the same Tables. The results shown in Tables 15 and 16 were obtained in the case of drying at 19°C and 20°\~30°C respectively.

The estimating temperature of the electrical resistance were made for all the sample at 17°C. Fig. 8 was derived by plotting the results in Tables 15 and 16.

From Fig. 8 it is observed that the electrical resistance of the raw fresh Atka mackerel meat decreased with the initial decreasing of the total amount of water-content during the drying of the fish meat at 19°C, and then it increased gradually at near the water-content at the point B.P. which indicates the blue change point by the cobaltous chloride method (Oyagi's method), and then it increased rapidly at the point of C.P. which indicates the constant weight of the sample during the drying at 30°C by that method. The results obtained from the electrical resistance of two samples of the Atka mackerel meat dried at 20°\~30°C are shown as almost the same curves.

The electrical resistance of the meat dried at 20°\~30°C was larger than that of the meat dried at 19°C at the same water-content in the range of the total water-conent of 60\~20%, that is, the higher the drying temperature is, the smaller the electrical resistance is.

Such a difference of the electrical resistance was considered to be caused by the difference of drying temperatures, because the estimating temperature of

Table 15. Relation between the electrical resistance (50 cycles) and the water-contents in fresh, salted and putrefied Atka mackerel meat (Drying temp., 19°C)

Fresh raw meat (A)		Salted meat (D)		Putrefied meat (F)		Putrefied meat (continue)	
Total Water (%)	Electrical Resistance ($K\Omega$)	T.W. (%)	E.R. ($K\Omega$)	T.W. (%)	E.R. ($K\Omega$)	T.W. (%)	E.R. ($K\Omega$)
79.6	0.45	70.2	—	79.1	0.455	15.1	50.0
70.3	0.41	64.2	0.035	74.0	0.340	14.5	135.0
48.4	0.45	55.2	0.045	68.6	0.450	14.0	160.0
38.2	0.65	52.0	0.049	60.9	0.400	12.9	279.0
24.5	5.50	45.6	0.060	49.6	0.420	12.4	300
20.2	60	40.0	0.110	37.4	0.500	10.2	7,000
14.8	1,000 over	31.0	0.260	29.4	1.23	—	—
—	—	21.2	65.0	17.0	3.23	—	—

Fresh raw meat (A)	Salted meat (D)	Putrefied meat (F)
Total Water 79.6%	Total Water 70.2%	Total Water 79.1%
Free Water 60.0%	Free Water 46.3%	Free Water 68.6%
at $B.P_1$ Bound Water 19.6%	at $B.P_2$ Bound Water 23.9%	at $B.P_3$ Bound Water 10.5%
at $B.P_1$ (g)* (0.961)	at $B.P_2$ (g)* (0.808)	at $B.P_3$ (g)* (0.503)
at $C.P_1$ (g)* (0.372)	at $C.P_2$ (g)* (0.358)	at $C.P_3$ (g)* (0.280)
	Ash content 16.6%**	
	NaCl content 15.7%**	

* g=gm of Bound Water per gm of dried matter.
** =% in dried matter.

Table 16. Relation between the electrical resistance (50 cycles) and the total water-contents in fresh and salted Atka mackerel meat (Drying temp., 20°~30°C)

Fresh raw meat (B)		Fresh raw meat (C)		Salted meat (E)	
Total Water (%)	Electrical Resistance ($K\Omega$)	T.W. %	E.R. ($K\Omega$)	T.W. %	E.R. ($K\Omega$)
78.8	0.60	78.4	0.52	70.7	—
70.2	0.45	71.6	0.40	52.0	0.050
61.7	0.47	64.8	0.38	49.6	0.065
54.1	0.60	60.9	0.46	48.4	0.070
43.1	0.95	50.2	0.72	45.6	0.075
34.6	1.00	43.9	1.50	44.3	0.120
28.2	4.20	30.5	1.60	39.1	0.130
20.9	8.50	27.1	8.00	33.5	0.220
18.0	70.0	25.8	18.0	25.4	0.66
17.4	360	21.9	70	22.1	1.35
16.8	700	17.4	1,000	20.2	76
12.8	6.500	—	—	16.6	270
—	—	—	—	14.7	3,000
—	—	—	—	11.45	8,000

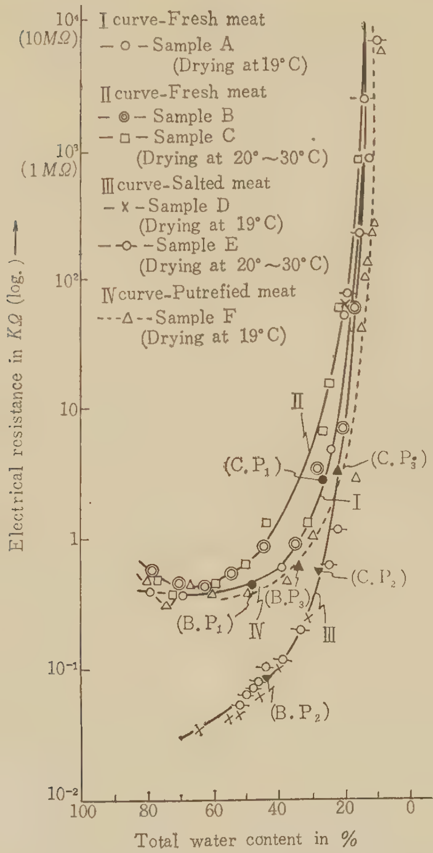


Fig. 8. Relation between the electrical resistance and the water-contents in fresh, salted and putrefied Atka mackerel meat

electrical resistance was the same after the maintenance of the samples in the desiccator at 17°C for some time.

As one of factors, pH influences the value of electrical resistance of the samples. The electrical resistance shows generally the maximum value at the isoelectric point of muscle protein. However, the value of pH of the fish meat is not considered to vary so much with the difference of the drying temperatures which were employed in this experiment (the average difference of temperature is 5°C). The electrical resistance may better be considered to be affected by the difference of the construction of muscle tissues originated by the drying temperatures. The muscle tissue of the fish meat dried at 19°C is rather homogeneous and fine, since the evaporation of water from the meat surface was considered to be slow, but the muscle tissue dried at 20°~30°C has some gaps because of the more rapid evaporation. Therefore the electrical conductivity decreases and the electrical resistance increases in the fish meat dried at higher temperature. But,

the ascending of the curve of the electrical resistance of the sample dried at 20°~30°C agrees with the sample dried at 19°C at below 20% of the total water-content, so it is supposed that there is rather little change of the amount of Bound Water in the two samples of fish meat dried at 19°C and 20°~30°C.

In the putrefied fish meat, the property of the curve of the electrical resistance of the sample is the same as in the raw fresh meat. The electrical resistance began to increase at the water-content of the point of $B.P_3$ by the cobaltous chloride method, and increased rapidly at the point of $C.P_3$. However, the curve of the putrefied fish meat slips wholly away from the curve of the raw fresh fish meat as expected from the preliminary experiment. Therefore, the values of electrical resistance of the putrefied fish meat were lower than those of the raw fresh meat in comparison with the same water-content. Furthermore the amount of Bound Water of the putrefied fish meat at the points of $B.P_3$ and $C.P_3$ was less than that of the raw fresh fish meat at $B.P_1$ and $C.P_1$. This fact perhaps shows the decreasing of Bound Water in the meat owing to the decomposition or destruction of the muscle tissue.

In the salted fish meat, no remarkable difference was observed in the electrical resistance of the samples dried at 19°C or at 20°~30°C. The salted fish meat contains NaCl as the electrolyte (about 16% of the dried matter of the sample). Its electrical resistance, therefore, is less than that of the raw fresh fish meat in the beginning of the estimation, but it increased rapidly during the drying, and at last the ascending of the curve agreed almost with the curve of the raw fresh meat.

As to the concentration of NaCl in the fish meat, such amount of NaCl, 16% per dried matter as above stated is enough to the ability of osmotic dehydration, therefore, a part of water which has weak binding strength with fish meat protein hydrates with NaCl, and less amount of Bound Water remains with the strong binding ability in the fish meat protein. Consequently, the electrical resistance was considered to increase rapidly by the existence of such Bound Water remained in the course of the drying.

(3) *Conclusions as to the results of estimations of the amounts of Bound Water in the various states of meat of various kinds of fish by the cobaltous chloride method and by the measurement of the electrical resistance*

The author has estimated the amount of Bound Water in the meat of various kinds of fish by the cobaltous chloride method (Oyagi's method)[14b] and by the measurement of the electrical resistance, and the following results and considerations were obtained.

(1) The amounts of Bound Water in the fresh raw meat of 14 kinds of fish were estimated; they differed with the kind of fish and individual bodies used, but it was recognized that the maximum amount of Bound Water is 55~82 gm, the minimum amount of Bound Water is 10~48 gm per 100 gm of the dried matter of the sample.

(2) The absolute amount of Bound Water per dried matter of fish decreased during the drying of fish meat. In this case, the amount of Free Water in the total amount of water in fish meat decreases from the beginning with the

drying of the meat. But the amount of Bound Water increased reciprocally to the decreasing of the amount of Free Water accompanying with the initial decreasing of the total amount of water in fish meat. The maximum value of the amount of Bound Water is observed at 40~50% of the total amount of water in fish meat, and then the amount of Bound Water decreased with the progressing of the drying of fish meat.

(3) The total amount of water in fish meat decreased somewhat by osmotic dehydration during the salting. In this case, the amount of Free Water also decreased, but that of Bound Water increased reciprocally. However, the change of the absolute amount of Bound Water per gm of the dried matter was not clearly recognized since some loss of dried matter caused by dissolving of soluble protein occurs as an accompanying phenomenon. But, the absolute amount of Bound Water is considered generally to decrease.

(4) In the case of the decomposition of fish meat, *e.g.*, in the putrefaction or in the fermentation of squid meat (Soused squid meat), it was observed that the total amount of water increased gradually, and the amount of Free Water increased also, while that of Bound Water decreased reciprocally. These results were observed to be different from the results influenced by dehydration during the drying or salting. But in this case, the change of the absolute amount of Bound Water was not clearly recognized by the cobaltous chloride method.

(5) During the autolysis or the putrefaction of fish meat, the electrical resistance decreased rapidly. This fact is considered to be due to the destruction of the muscle tissue of the fish meat. And it was also considered that the larger the amount of Bound Water in fish meat was, the higher the electrical resistance was. From this consideration, the amount of Bound Water was considered to decrease with putrefaction.

(6) From the estimated results of the electrical resistance at various water-contents in the fresh raw fish and salted fish meat, it was supposed that when a large amount of NaCl penetrates into the fish meat, a part of the amount of Bound Water, except Bound Water possessing strong combining strength, hydrates with NaCl penetrated.

III. THE INFLUENCE OF BOUND WATER UPON THE VELOCITY OF DRYING OF FISH MEAT

As stated in a previous article, Higuchi[12)] has estimated the amounts of Bound Water in squid and flat fish meat. When samples of those fish meats were dried, the amount of Free Water decreased and that of Bound Water relatively increased with the decreasing of the total amount of water.

The present author has also studied the distribution of the amount of Bound Water in the raw or dried meat of various kinds of fish as described in the previous Section II. According to his results, the proportion of the amount of Bound Water to the total amount of water in those fish meats indicated 30% (minimum) ~95% (maximum) in the range of 50~15% of the total amount of water-content.

The fact that those values of amount of Bound Water in the dried fish meats are much larger than those of the fresh raw fish meat indicates that when fish meat is drying, the amount of Free Water at first decreases while that of Bound Water increases relatively.

On the physical problems concerning the drying of fish meat, Fujiwara, Tsuda and Yasui[27], Inoue[28], and recently Kawakami[29] have studied and discussed various aspects. In general, drying period of common granular solid matter, *e.g.* sand or clay, may be divided clearly into three stages: (1) the period of constant rate of drying, (2) the period of first stage of falling rate of drying and (3) second stage of the same. In the drying of fish meat, *e.g.* in the case of washed fish meat or fish meat moistened with water which has a thin layer of water regarded as a true free water, as long as this thin layer of water continues to evaporate, the period of constant rate of drying may be admitted. However, in the case of drying of fish meat having no thin layer of water in the surface it is considered that already in the initial of drying of fish meat, the mechanisms of drying corresponds to the period of first stage of falling rate of drying stage. This period of first stage of falling rate of drying appears to continue to the last of the drying without clear difference between the first stage and second stage of falling rate of drying, because of the hydrophilic properties of fish protein. Kawakami[29] has pointed out that the amount of Bound Water in the fish meat has important influence upon the mechanism of drying.

The present author has estimated the amount of Bound Water in the various kinds of fish muscle by means of the vapour tension method and the cobaltous chloride method; he offers a discussion of the thermodynamic properties of water in the fish meat having various amounts of water-content. He has also determined the drying curve of fish meat at the low drying temperatures (35°~40°C), and discussed the influence of the amount of Bound Water upon the velocity of drying.

1. Experimental part

(1) Samples

As samples, 12 kinds of fish, mollusc and crustacean were employed, such as crucian carp (*Carassius auratus* (LINNE)), "Soi" in Japanese (a kind of gray rock cod) (*Sebastichthys trivitatus* (HIRGENDORF)), yellow-tail (*Seriola quinqueradiata* TEMMINCK *et* SCHLEGEL), Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ), sardine (*Sardinia melanosticta* (TEMMINCK *et* SCHLEGEL)), mackerel (*Scomber japonicus* HOUTTUYN), tuna (*Thunnus orientalis* (TEMMINCK *et* SCHLEGEL)), "Umitanago" (a species of minnow) (*Ditrema temminckii* BLEEKER), "Magarei" (Flat fish) (*Limanda herzensteini* JORDAN *et* SNYDER), "Hirame" (Flat fish) (*Paralichthys olivaceus* (TEMMINCK *et* SCHLEGEL)), squid (*Ommastrephes sloani pacificus* STEENSTRUP), "Botan-ebi" (a species of prawn) (*Pandalus hyposinotus* BRANDT). As samples there were taken: in the case of fish, about 5 gm of central back part of the fish meat above lateral line, in the case of squid about 5 gm of back part of the body, in the case of prawn about 5 gm of body meat which was removed from crust. From 5 gm of those various kinds of meat, about 1~2 gm were estimated by the vapour tension method, and about 1 gm each was employed for drying at 40°C.

(2) The vapour tension method

The vapour tension method was the same as that employed in the previously described in Paper I, Experiment III, 1, (1), (III)[1a]. The temperature at which estimates were made differed with the kind of samples; its range was 15°~21°C.

(3) Cobaltous chloride method

Cobaltous chloride method was that of Oyagi which is the same as that used in the previously described in Paper I, Experiment III, I, (II), (B)[1a].

By this method the maximum amount of Bound Water at B.P. during the drying of dyed samples at 25°~30°C was estimated.

(4) Experimental method

For the various samples as above described, the relation between the water-activity "a" of the samples (that is the relative vapour pressure, p/p_0), and various amounts of water-content, "g", (gm of water per gm of dried matter) was determined by the vapour tension method; also the amount of Bound Water was estimated by the cobaltous chloride method.

On the other hand, slices of the samples about 1 gm in weight were made, and dried in a drier at 35°~40°C. Then they were weighed after taken from the drier at certain definite intervals. From data thus obtained, the relation between the drying time, "t", and water-content, "g", was determined.

Here, the drying temperature was decided to be 35°~40°C in order to avoid the heat denaturation (coagulation) of fish protein. The estimation of the amount of Bound Water in fish meat by the cobaltous chloride method is possible at a lower temperature such as 25°~30°C. Therefore, when the drying of fish meat was made at below those temperatures it is feared that the drying of fish meat reaches to equilibrium while still containing an amount of water greater than that regarded as the amount of Bound Water estimated by the cobaltous chloride method. Therefore it was considered that there was need of increasing the thermodynamic energy by keeping the drying temperatures at 35°~40°C.

2. Experimental results and discussion

The results obtained are shown in Tables 17~28 and Figs. 9~20. In every Table, there are shown the relation between the absolute amount of water-content, "g", obtained by the vapour tension method for each sample and the water-activity of samples "a"; the relation "a" and "$p/g(p_0-p)$" and the relation between the amount of water-content, "g", and drying time, "t" (hrs), under constant drying at 40°C. The values of the amount of Bound Water obtained by the cobaltous chloride method are shown in the lower margin of the tables. The relation between "a" and "$p/g(p_0-p)$" was derived from B.E.T. equation (3) which is offered for the multimolecular layer's adsorption by Brunauer, Emmett and Teller[23a] in 1938. The reason for applying B.E.T. equation (3) is that the curve of "$g-a$" (or "$g-p/p_0$") is regarded as a kind of desorption isotherm (that is, isothermal curve for desorption of water) as stated in earlier Paper II[1b].

$$p/g(p_0-p)=1/g_mC+\frac{C-1}{g_mC}\times(p/p_0) \qquad (3)$$

where g_m and C are B.E.T. constants.

In Figs. 9~20, each curve I shows the relation for each sample between the absolute amount of water-content "g", estimated at definite estimating temperature and the water-activity "a" (that is, p/p_0). Each curve II shows the relation between "p/p_0" and "g/a". Each straight line III shows the linear relation between "p/p_0" and $p/g(p_0-p)$ derived by using B.E.T. equation (3). Each curve IV shows the relation between drying time "t" under the drying at 40°C, and the amount of water-content "g".

Table 17 and Fig. 9 show the experimental results for crucian carp (*Carassius auratus* (LINNE)). As seen by curve IV in Fig. 9, the amount of water-content decreased nearly in a straight line from 0 to 6 hours' drying, then the rate of decrease was small from 6 to 10 hours, and the drying reached nearly to equilibrium at from 11 to 12 hours. In this drying, as the surface area of the dried fish meat has not been estimated, the rate of decrease of water-content for the drying time "t", "G/t" (where G is the amount of water evaporated per hour) was calculated, and when the relation of "G/t" for every period of drying was observed as drying velocity per hour, the decreasing was seen to be almost exponential function. From this, it is clear that after the initial period of drying, the drying mechanism follows the first stage of falling rate of drying; the difference between the first and second stages of falling rate of drying was

Table 17. Crucian carp meat

Estimation of vapour tention at 15°C			Drying at 40°C	
g*	p/p_0 (or "a")	$p/g(p_0-p)$	Drying time "t" (hrs.)	Water-content g*
1.98	0.932	7.45	0	4.45
0.65	0.891	12.55	1.0	3.28
0.23	0.597	6.45	2.0	2.44
0.17	0.400	3.92	3.0	1.71
0.13	0.300	3.23	4.0	1.16
0.11	0.200	2.18	5.0	0.82
0.10	0.150	1.75	6.0	0.55
0.08	0.100	1.39	7.0	0.36
0.055	0.04	0.76	8.0	0.25
—	—	—	9.0	0.186
—	—	—	10.0	0.156
—	—	—	11.0	0.152
—	—	—	12.0	0.152

* g: Gm of water per gm of dried matter.
$g_{B.P.}=0.578$ (The amount of Bound Water at the point of B.P. by Oyagi's method).

Table 18. "Soi" meat

Estimation of vapour tension (20.5°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
2.49	0.978	18.7	0	3.98
1.93	0.963	14.1	1.0	2.70
1.48	0.936	10.1	2.0	1.94
1.15	0.889	6.96	2.5	1.68
0.79	0.863	7.97	3.0	1.42
0.53	0.813	8.19	3.5	1.24
0.32	0.675	6.51	4.0	1.04
0.24	0.600	6.25	4.5	0.89
0.21	0.520	5.19	5.0	0.75
0.15	0.450	5.44	6.0	0.54
0.11	0.350	4.99	7.0	0.34
0.09	0.263	3.98	8.0	0.24
0.08	0.200	3.13	9.0	0.21
0.075	0.150	1.58	10.0	0.195
0.070	0.100	1.44	11.0	0.191
0.060	0.064	1.15	12.0	0.191

$g_{B.P.}=0.447$

Table 19. Yellow-tail meat

Estimation of vapour tension (20.5°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
3.09	0.992	36.9	0	3.09
1.68	0.972	21.0	1.0	2.24
1.14	0.950	16.7	2.0	1.61
0.86	0.926	14.6	2.5	1.32
0.53	0.870	12.8	3.0	1.08
0.30	0.768	11.1	3.5	0.92
0.173	0.614	14.5	4.0	0.76
0.165	0.500	6.07	4.5	0.63
0.159	0.400	4.19	5.0	0.54
0.152	0.297	2.77	6.0	0.36
0.128	0.250	2.53	7.0	0.24
0.098	0.186	2.32	8.0	0.19
0.089	0.150	2.03	9.0	0.167
0.074	0.100	1.50	10.0	0.161
0.055	0.050	0.963	11.0	0.158
0.042	0.037	0.928	12.0	0.158

$g_{B.P.}=0.501$

Table 20. Atka mackerel meat

Estimation of vapour tension (20°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
3.97	0.989	21.2	0	3.99
2.48	0.978	18.1	1.0	2.55
1.77	0.971	18.4	2.0	1.76
1.31	0.946	13.3	3.0	1.18
0.94	0.910	10.6	4.0	0.75
0.69	0.852	8.32	5.0	0.47
0.53	0.797	7.39	6.0	0.29
0.21	0.572	6.28	7.0	0.195
0.14	0.400	4.77	8.0	0.155
0.10	0.263	3.56	9.0	0.153
0.08	0.150	2.20	10.0	0.151
0.07	0.107	1.60	11.0	0.151
0.06	0.050	0.88	—	—

$g_{B.P.}=0.442$

Table 21. Sardine meat

Estimation of vapour tension (18.5°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
3.30	0.992	40.3	0	3.30
2.33	0.990	42.7	0.5	2.69
1.64	0.985	41.7	1.0	2.23
1.31	0.970	24.9	1.5	1.91
1.10	0.962	23.2	2.0	1.41
0.83	0.950	23.0	2.5	1.19
0.71	0.923	16.9	3.0	0.99
0.59	0.910	18.1	3.5	0.88
0.49	0.857	13.1	4.0	0.76
0.37	0.769	9.22	4.5	0.66
0.28	0.580	4.93	5.0	0.59
0.25	0.499	3.98	6.0	0.44
0.24	0.413	2.94	7.0	0.31
0.22	0.376	2.73	8.0	0.22
0.21	0.280	1.86	9.0	0.16
0.19	0.251	1.77	10.0	0.115
0.18	0.145	0.945	11.0	0.099
0.16	0.084	0.577	12.0	0.095
0.15	0.076	0.549	13.0	0.095
0.145	0.056	0.407	—	—

$g_{B.P.}=0.608$

Table 22. Mackerel meat

Estimation of vapour tension (21°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
1.55	0.933	9.01	0	2.88
1.16	0.899	7.67	1.0	2.05
0.90	0.875	7.76	2.0	1.43
0.61	0.839	8.58	3.0	1.00
0.41	0.801	9.84	4.0	0.75
0.28	0.693	8.07	5.0	0.55
0.18	0.572	7.39	6.0	0.40
0.13	0.469	6.57	7.0	0.36
0.11	0.384	5.67	8.0	0.255
0.08	0.292	4.37	9.0	0.218
0.075	0.207	3.49	10.0	0.188
0.072	0.150	2.46	11.0	0.178
0.068	0.100	1.63	12.0	0.175
0.065	0.078	1.31	13.0	0.175
0.060	0.050	0.088	—	—

$g_{B.P.}=0.557$

Table 23. Tuna meat

Estimation of vapour tension (18.5°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
2.46	0.984	27.8	0	2.54
1.45	0.952	13.4	1.0	1.70
1.03	0.923	11.5	2.0	1.18
0.76	0.893	10.9	3.0	0.85
0.54	0.835	9.38	4.0	0.64
0.33	0.738	8.59	5.0	0.45
0.26	0.650	7.16	6.0	0.32
0.22	0.553	5.04	7.0	0.19
0.17	0.450	4.81	8.0	0.144
0.16	0.344	2.74	8.5	0.125
0.145	0.226	2.01	9.5	0.116
0.140	0.150	1.26	10.5	0.110
0.130	0.098	0.838	11.5	0.107
0.120	0.043	0.375	12.5	0.107

$g_{B.P.}=0.631$

Table 24. "Umitanago" meat

Estimation of vapour tension (20.5°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
2.23	0.965	12.6	0	3.55
1.56	0.955	14.2	1.0	2.06
0.89	0.911	11.6	1.5	1.60
0.54	0.800	7.41	2.0	1.19
0.39	0.714	6.44	2.5	0.89
0.24	0.600	6.25	3.0	0.67
0.18	0.468	4.91	3.5	0.50
0.16	0.400	4.17	4.0	0.36
0.15	0.300	2.85	4.5	0.25
0.13	0.200	1.93	5.0	0.19
0.12	0.150	1.51	6.0	0.155
0.11	0.118	1.22	7.0	0.141
0.08	0.050	0.66	8.0	0.136
0.035	0.035	1.04	9.0	0.136

$g_{B.P.}$....not estimated.

Table 25. "Magarei" meat

Estimation of vapour tension (21°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
1.89	0.960	12.8	0	4.38
1.22	0.918	9.18	1.0	2.82
0.805	0.871	8.38	2.0	1.64
0.507	0.778	6.88	3.0	0.96
0.315	0.660	6.16	4.0	0.61
0.187	0.536	6.15	5.0	0.36
0.108	0.292	3.82	6.0	0.22
0.093	0.250	3.58	7.0	0.15
0.084	0.200	2.98	7.5	0.144
0.073	0.131	2.05	8.0	0.141
0.059	0.065	1.18	9.0	0.141

$g_{B.P.}=0.477$

Table 26. "Hirame" meat

Estimation of vapour tension (21°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
1.75	0.972	20.8	0	3.74
1.05	0.964	24.9	1.0	2.36
0.52	0.876	13.5	2.0	1.50
0.23	0.652	8.09	3.0	0.95
0.17	0.500	5.88	4.0	0.63
0.15	0.400	3.76	5.0	0.37
0.13	0.300	3.29	6.0	0.22
0.12	0.200	2.07	7.0	0.155
0.11	0.168	1.82	8.0	0.144
0.10	0.100	1.11	9.0	0.139
0.07	0.041	0.60	10.0	0.134
—	—	—	11.0	0.134

$g_{B.P.}=0.541$

Table 27. "Botan-ebi" meat

Estimation of vapour tension (20.5°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
3.53	0.991	30.9	0	3.53
1.07	0.897	8.17	2.0	2.28
0.75	0.844	6.74	3.0	1.75
0.49	0.749	6.08	4.0	1.26
0.32	0.666	6.23	5.0	0.91
0.22	0.544	5.42	6.0	0.69
0.13	0.388	4.88	7.0	0.50
0.08	0.184	2.81	8.0	0.38
0.07	0.150	2.50	9.0	0.29
0.05	0.100	2.12	10.0	0.226
0.045	0.050	1.17	11.0	0.184
0.040	0.030	0.93	12.0	0.162
—	—	—	13.0	0.162

$g_{B.P.}=0.574$

Table 28. Squid meat

Estimation of vapour tension (20°C)			Drying (40°C)	
g	p/p_0 (or "a")	$p/g(p_0-p)$	t	g
2.73	0.988	29.7	0	2.94
1.79	0.974	21.6	1.0	2.22
1.34	0.947	13.3	2.0	1.76
1.07	0.920	10.7	2.5	1.63
0.84	0.904	11.4	3.0	1.48
0.69	0.893	12.2	4.0	1.21
0.59	0.853	9.76	5.0	1.01
0.50	0.842	10.4	6.0	0.76
0.41	0.799	9.61	7.0	0.61
0.35	0.774	9.85	8.0	0.51
0.29	0.742	9.02	9.0	0.39
0.24	0.673	8.44	10.0	0.33
0.21	0.635	8.32	11.0	0.25
0.174	0.573	7.71	12.0	0.21
0.148	0.559	7.78	13.0	0.18
0.124	0.464	6.97	14.0	0.156
0.108	0.443	7.36	15.0	0.137
0.078	0.398	8.48	16.0	0.123
0.064	0.289	6.44	17.0	0.108
0.043	0.186	5.33	18.0	0.100
0.038	0.150	4.23	19.0	0.100
0.032	0.105	3.76	20.0	0.100
0.030	0.050	1.77	—	—

$g_{B.P.}=0.729$

not clear in the final period of drying. Here, supposing these drying mechanisms of fish meat at 40°C apply to the equation (4) of slow drying[27],

$$W = W_1 e^{-\lambda N} \qquad (4)$$

where, W_1 : the weight of sample before drying (gm)
W : the weight of sample after the drying (gm)
N : drying time "t", (hours)
λ : drying coefficient (gm/hrs)

The values of drying coefficient for crucian carp meat calculated from equation (4) are shown in the 12th~14th columns of Table 29. The value of λ_1 was 0.244 in the range of 0~8 hrs. of drying time, the value of λ_2 was 0.025 in the range of 8~10 hrs. The proportion of λ_2 to λ_1 ($\lambda_2/\lambda_1 \times 100\%$) was 10.2%. For various other kinds of samples, the proportions of λ_2 to λ_1 (%), ranged 10~18% as shown in Table 29.

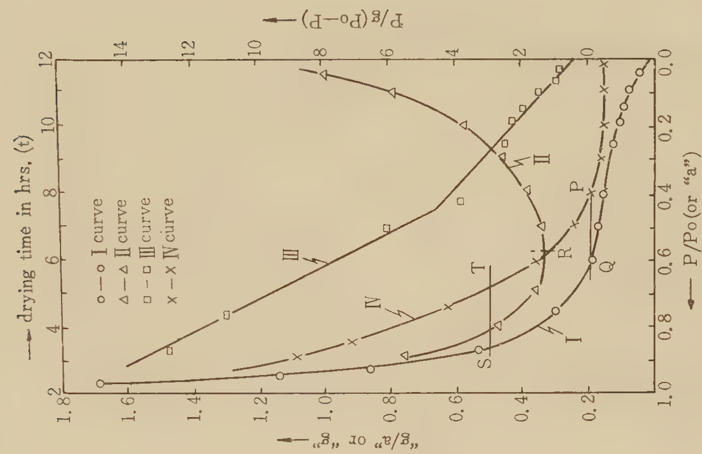


Fig. 11. Yellow-tail meat

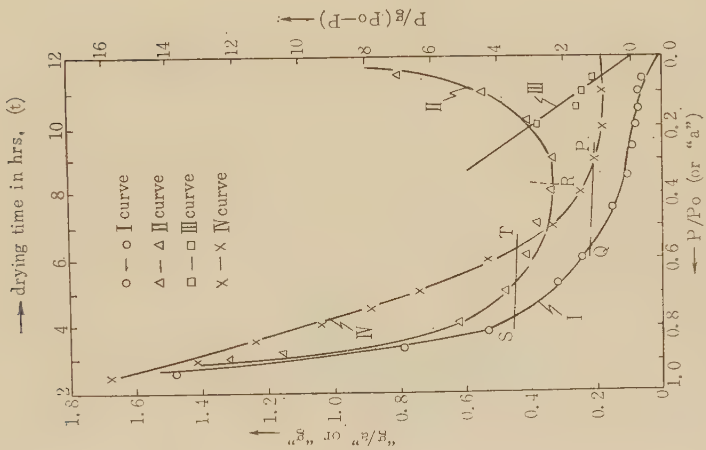


Fig. 10. "Soi" meat

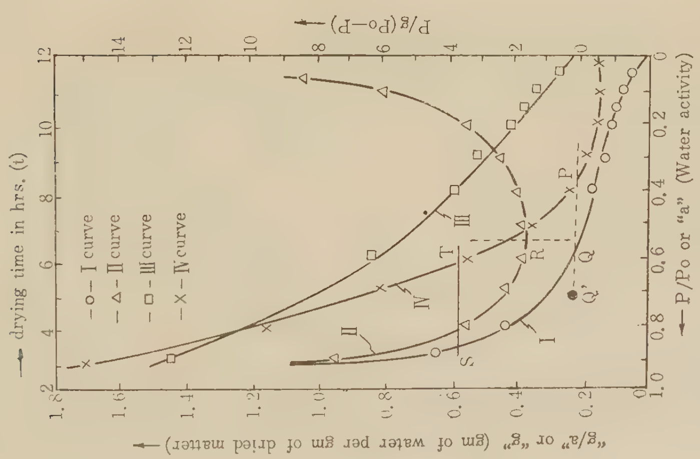


Fig. 9. Crucian carp meat

I curve $(g-p/p_0)$, II curve $(p/p_0-g/a)$, III curve $(p/p_0-p/g(p_0-p))$, IV curve $(g-t)$
(Similar signs in subsequent figures show the same mean as in these figures.)

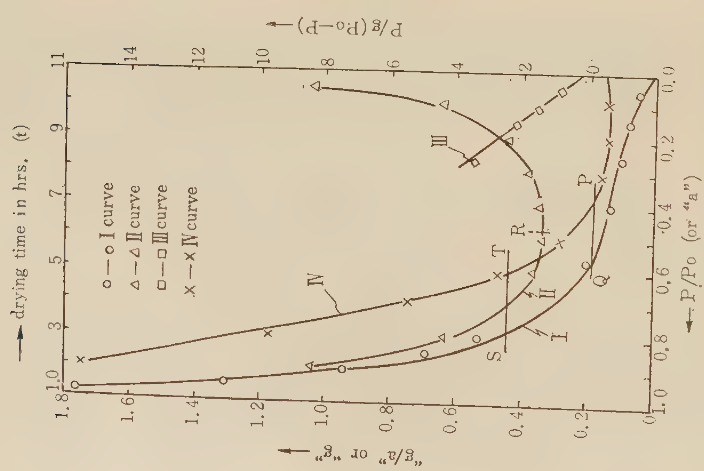


Fig. 12. Atka mackerel meat

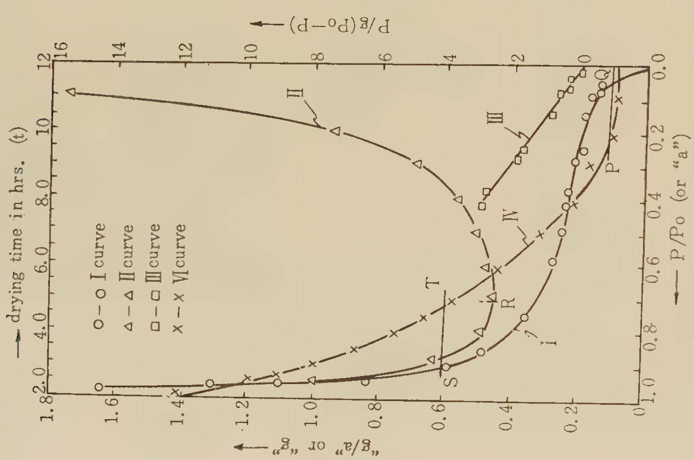


Fig. 13. Sardine meat

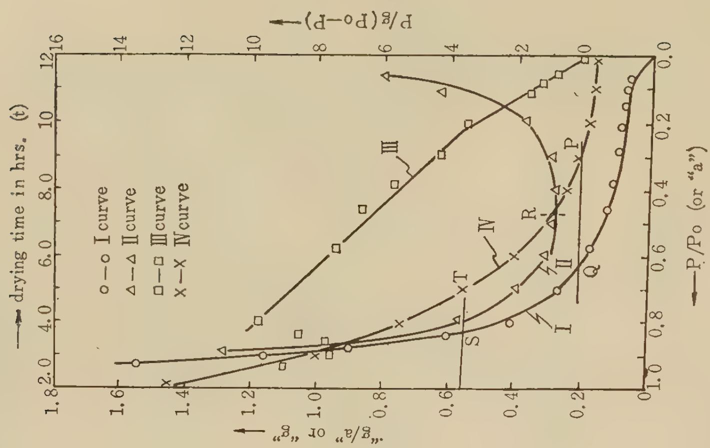


Fig. 14. Mackerel meat

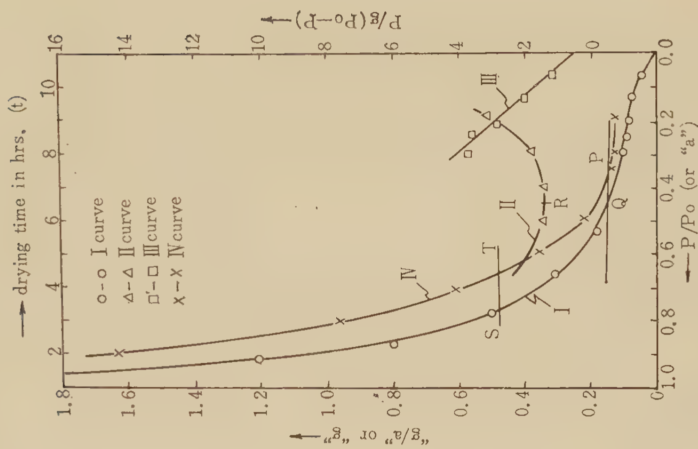


Fig. 17. "Magarei" meat

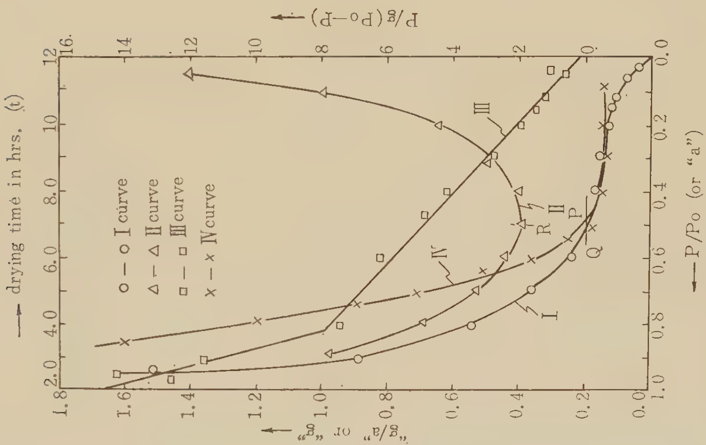


Fig. 16. "Umitanago" meat

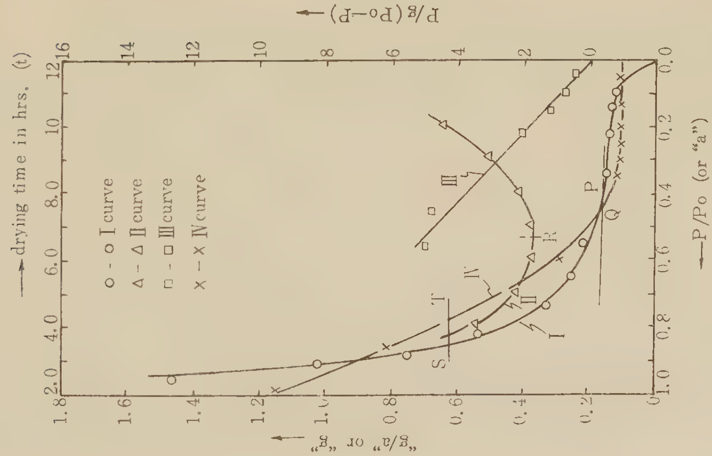


Fig. 15. Tuna meat

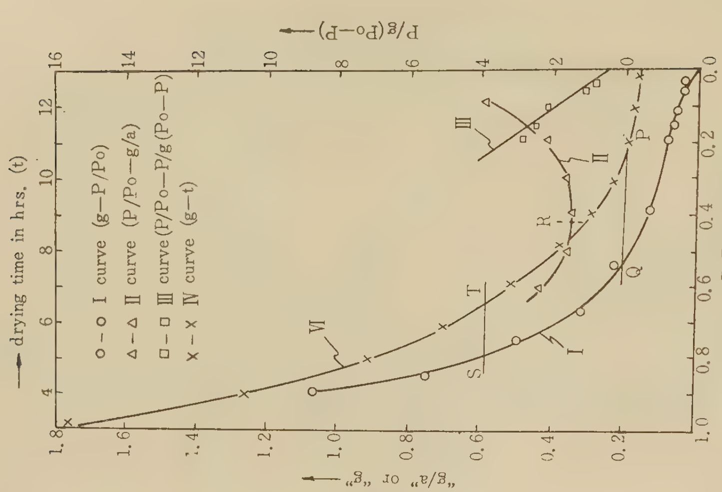


Fig. 20. "Botan-ebi" meat

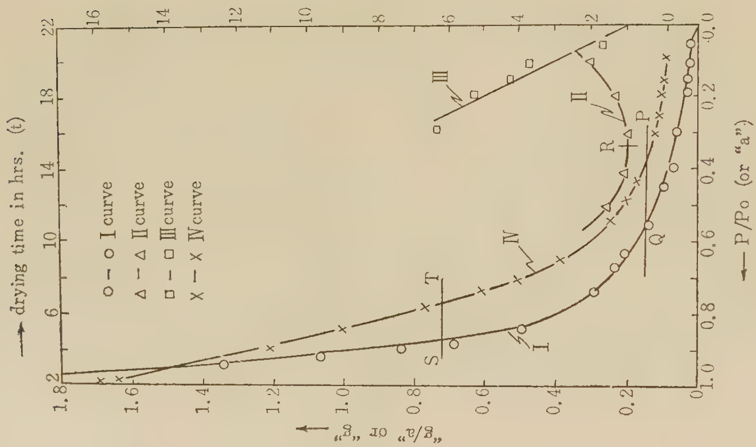


Fig. 19. Squid meat

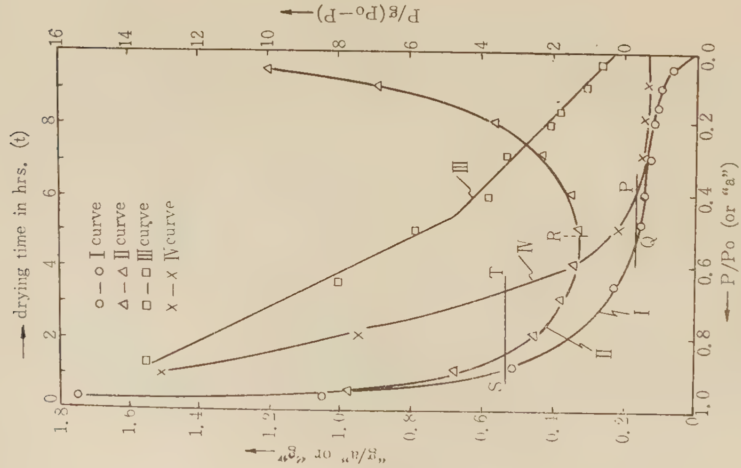


Fig. 18. "Hirame" meat

Table 29. The influence of Bound Water upon the velocity of drying of fish meat

Sample	Est. temp. (t°C)	B.E.T. constant		g'_m	"a" at g'_m	$\frac{g'_m}{g_m}$	E_1-E_L Kcal/mol	g_P	"a" at $(g_P)t_{exp.}$°C	"a" at $(g_P)_{t=40°C}$	Drying coefficient, λ		
		C	g_m								λ_1	λ_2 at g_P	$\frac{\lambda_2}{\lambda_1}\times100$ %
Crucian carp	15	25	0.108	0.208	0.545	1.93	1.8	0.218	0.575	0.739	0.224 (0~8 hrs.)	0.025 (8~10 hrs.)	10.2
"Soi"	20.5	15.5	0.0645	0.130	0.390	2.02	1.6	0.210	0.530	0.629	0.183 (0~7 hrs.)	0.0195 (7~9 hrs.)	10.7
Yellow-tail	20.5	14	0.107	0.185	0.580	1.73	1.5	0.190	0.600	0.704	0.171 (0~7 hrs.)	0.029 (7~9 hrs.)	16.8
Atka mackerel	20.0	53	0.0755	0.160	0.470	2.12	2.3	0.195	0.550	0.707	0.223 (0~6 hrs.)	0.032 (7~9 hrs.)	14.4
Sardine	18.5	108 ?	0.142	0.31	0.680	2.18	2.6	0.11	0.04	0.055	0.157 (0~8 hrs.)	0.023 (9~11 hrs.)	14.6
Mackerel	21	271 ?	0.0613	0.120	0.420	1.96	3.3	0.215	0.634	0.658	0.141 (0~8 hrs.)	0.025 (8~10 hrs.)	17.7
Tuna	18.5	15	0.098	0.180	0.560	1.84	1.6	0.170	0.40	0.484	0.142 (0~8 hrs.)	0.024 (7~9.5 hrs.)	16.8
"Umitanago"	20.5	10	0.095	0.198	0.500	2.08	1.3	0.190	0.500	0.575	0.267 (0~5 hrs.)	0.0276 (5~6 hrs.)	10.3
"Magarei"	21	15	0.078	0.148	0.450	1.90	1.6	0.150	0.460	0.531	0.253 (0~6 hrs.)	0.031 (0~6 hrs.)	12.2
"Hirame"	21	44	0.0100	0.165	0.500	1.65	2.9	0.170	0.500	0.536	0.226 (0~6 hrs.)	0.0298 (6~8 hrs.)	12.8
Squid	20	22	0.046	0.08	0.350	1.72	1.8	0.140	0.520	0.633	0.098 (0~12 hrs.)	0.016 (12~18 hrs.)	16.3
"Botan-ebi"	20.5	38	0.073	0.138	0.400	1.89	2.1	0.190	0.515	0.544	0.139 (0~9 hrs.)	0.025 (10~12 hrs.)	18.0

The values of drying coefficient, λ_1, decreased remarkably (10~18%) at the turning point of "p" on the "$t-g$" curve (curve IV) in Fig. 9 showing the drying curve for crucian carp meat (and other samples) and then reached to the state of drying equilibrium.

The author has considered the turning point on the curve IV, "P", the boundary between the first stage and the second stage of falling rate of drying.

The value of the amount of water-content at the turning point, g_P, (the gm of water per gm of dried matter) is 0.218 for crucian carp meat as shown in the 9th column of Table 29. The values for other samples were 0.14~0.215, except for the value of sardine at 0.11.

In order to transfer the turning point, "P", of curve IV onto the curve I ($g-a$ curve), a parallel line was drawn from the point of "P" with the abscissa in Fig. 9 (or in other Figs.), then this parallel line crossed with curve I. The intersection is the point of "Q".

The value of "a" at the point of "Q" for crucian carp was 0.575 as shown in the 10th column of Table 29. The values of "a" for other samples were 0.4~0.65 excepting the value for sardine at 0.04. From those Figs., the values of drying coefficient of the final period of drying at 40°C decreased to 10~18% of those coefficient of the initial period as a boundary with the amount of water-content "g_P" at 0.4~0.65 of the values of "a".

In Fig. 9 and other Figs., curves I and IV were divided by the T–S line. The intersections ("T" and "S") indicate $g_{B.P.}$ showing the amount of Bound Water estimated by the cobaltous chloride method. The point of "T" or "S" shows the point at which pink coloured samples dyed with cobaltous chloride solution become blue. The value of "a" at the point of "S" on curve I in Fig. 9 was about 0.87 for crucian carp meat. The values of "a" for other samples were in the range of 0.75~0.95. That is to say, points of "S" in Figs. were situated in the range of 0.75~0.95 of "a". From those results it is considered that the amount of Bound Water estimated by the cobaltous chloride method "$g_{B.P.}$" seems to be larger than the water-content at respective points "Q" or "P" on curves I or IV, and the value of "a" for the values of "$g_{B.P.}$" seems to be larger than the value of "a" for the value of "g_P" estimated at the point of "Q".

In curve of "$p/p_0-g/a$" (curve II) as seen in Fig. 9 and other Figs., the value of "g/a" decreased with the decreasing of value of "p/p_0", that is to say, it decreased with the decreasing of the amount of water-content in the initial period of drying, and the value of "g/a" showed the minimum value at the point, "R", on curve II.

However, on the contrary, the value of "g/a" increased below the value of "a" corresponding to the point of "R".

The value of the amount of water-content "g" which shows the value of "a" corresponding to the point of "R" was determined from curve I, and expressed as "g'_m" as shown in the 5th column of Table 29. This value of "g'_m" for crucian carp was 0.208, those for other samples were about 0.12~0.20 excepting the values of "g'_m" for sardine, 0.31, and for squid, 0.08.

Those values of "g'_m" are somewhat smaller than the value of "g_p" corresponding to the point of "P" which is the turning point of curve IV or corresponding

to the point of "Q" on curve I, but the values of the former are comparatively similar to that of the latter. The value of "a" corresponding to the value of "g'_m" for squid was the minimum, 0.350, and that for sardine was maximum, 0.680, as shown in column 6 of Table 29. The values for other samples were naturally in the range between the minimum and maximum values above stated.

As seen in curve III of Fig. 9 and other Figs., the relation between the values of "p/p_0" and "$p/g(p_0-p)$" is found clearly to be linear. But in Fig. 9 showing the crucian carp meat, the straight line curve III is divided into two parts, and the inclinations of those two parts (two straight lines) are different in the ranges of the value of "a", 0~0.5 and 0.6~0.9.

This change in inclination means the appearance of a new activated surface on the adsorption layer at the adsorption of water molecule. In the case of crucian carp meat, the value of constant, "g_m", in equation (3) calculated from the relation of the straight line showing the value of "p/p_0" to be in the range of 0~0.5 in the initial process of adsorption, was 0.108 (per gm of dried matter) as listed in column 4 of Table 29. The value of "g_m" in this case means the amount of water required to cover the surface of fish meat protein with one molecule of water layer.

For the various other samples, the flat fish ("Magarei") was 0.078 (minimum value), and the sardine was 0.142 (maximum). That is to say the values of "g_m" distributed in the range of 0.07~0.15.

As previously stated, the value of "g'_m" an amount of water-content corresponding to the value of "a" at the point of "R" on curve II, was found to be 0.208 for crucian carp meat. The values of the ratio of g'_m to g_m, "g'_m/g_m", are shown in 7th column of Table 29. The value for crucian carp meat was 1.93. For the various other kinds of samples, the values of the ratio were 2.18 for sardine (maximum), 1.65 for "Hirame" meat (minimum), and 1.92 on the average.

According to the results obtained for various poly-molecular compounds by Bull[22], the value of "g'_m/g_m" (as the value of v'_m/v_m was expressed in his study) was 2 on the average. He has pointed out that the adsorption process of the first layer is complete at "g_m" of the water-content, and the adsorption of the second layer continues to the water-content of "g'_m", while at above the value of "g'_m", the most part of water keeps liquid state, and it comes to have the properties of a true solution.

In conclusion, it may be said that the amount of water-content, "g_P" at the turning point of "P" on the drying curve at 40°C (curve IV) are somewhat larger than the value of "g'_m" of the water-content showing the value of "a" corresponding to the point of "R" on curve II, but the two values are almost similar. The values of "a" at "g_P" of the water-content were about 0.40~0.64. However, strictly speaking, as the process of water evaporation from the sample continues with thermodynamic energy corresponding to 40°C of the drying temperature, the value of "g_P" at the turning point "P", on drying curve IV must be transferred onto the "$g-p/p_0$" curve which may be obtained at 40°C.

Generally the "$g-p/p_0$" curve slips down to the value of 1.0 (to the left side), with the rising of estimating temperature, in other words, the value of "a" at a

certain water-content slips down to the value of 0 (to the right side) with falling of the temperature. The values of curve I in Figs. 9~20 were estimated within the temperature range of 15°~21°C, therefore, point "Q" on curve I ought to decline to the left side in the case of the estimation at 40°C. As it was impossible to estimate at above 25°C owing to the less capacity of manometer of the vapour tension apparatus, the estimation of vapour pressure at 40°C has not been done.

In order to know the slipping degree of the point "Q", the values of "C", a constant concerned with the heat of adsorption in B.E.T. equation (3), were calculated from the linear relationship of the values of p/p_0 to $p/g(p_0-p)$, and these values obtained are shown in column 3 of Table 29 for every kind of samples.

According to B.E.T. theory, this constant value "C" is expressed as follows,

$$C=a_1 g/b_1 \exp(E_1-E_L)/RT \qquad (5)$$

where, "a_1" and "b_1" are constants concerned with water-condensation and evaporation properties on the first layer of adsorption surface: "g" is the ratio of both constants at above second layer, b_i/a_i, in which "i" is the number of adsorption layers, that is,

$$i=2, 3, 4, \ldots\ldots n \ldots\ldots$$

Supposing that, for the simplify

$$g=b_i/a_i$$

then, $a_1=a_i$ and $b_1=b_i$

therefore, equation (5) becomes

$$C=\exp(E_1-E_L)/RT \qquad (6)$$

From equation (6), E_1-E_L, an adsorption heat of water in the sample under consideration, can be calculated, where E_1 is the adsorption heat of the first layer, and E_L is the heat of liquefaction of pure water, that is 9.7 Kcal/mol. As shown in column 8 of Table 29, the values of (E_1-E_L) for each sample are in the range of 1.3~3.3 Kcal/mol.

As reported in a previous Paper II[1b], the author has determined the curve of "$g-a$" at 13° and 8°C for gelatine and studied the relation between the change of the differential molal enthalpy, $\Delta\bar{H}$, at the adsorption and the values of "a"; according to the results obtained, the value of $\Delta\bar{H}$ was above 10 Kcal/mol in the range of 0.2~0.0 of "a", 3~7 Kcal/mol in the range of 0.2~0.7 of "a" and with the progressing of adsorption, the value of $\Delta\bar{H}$ decreased rapidly to 0~2 Kcal/mol at above 0.7 of "a". In that case the value of (E_1-E_L) was obtained as 3.8~3.9 Kcal/mol for gelatine. The value of (E_1-E_L) of fish meat obtained in this experiment is less than that of gelatine, so the value of $\Delta\bar{H}$ of fish meat is also supposed to be somewhat less than that of gelatine. In the range of 0.4~0.65 of "a" at point "Q" corresponding to point "g_P" in Fig. 9 and other Figs., the value of $\Delta\bar{H}$ is considered to be less than 3~4 Kcal/mol, so the values of (E_1-E_L) for each sample in Table 29 may be regarded as the values of $\Delta\bar{H}$ without much difference between them. Therefore, in the following equation (7) of Clausius Clapeyron, if the values of "a", corresponding to point "Q" on curve I of each sample at various estimating temperature T_1, are known, and supposing the value of $\Delta\bar{H}$ equals with the value of (E_1-E_L); then the values of a_2 corresponding

to 40°C of estimating temperature (T_2=273.1+40=313.1°K) can be calculated.

$$\Delta\overline{H} = -\frac{RT_1T_2}{T_1-T_2} = \ln a_2/a_1 \qquad (7)$$

The results of calculation thus obtained are shown in the 11th column of Table 29.

The value of "a" corresponding to point "Q" in Fig. 9 which was 0.575 for crucian carp meat was recognized to change to 0.739 of the value of "a" corresponding to point Q' at 40°C estimating temperature. Similarly, the value of "a" for other samples was 0.484 for tuna meat (minimum) while the maximum value was 0.739 for crucian carp, as above stated, except the value of sardine at 0.055. These values correspond to the point of "Q'". That is to say, the point of Q' seems to distribute in the range between the 0.48~0.74 value of "a". However, as a general characteristic of the "g—a" curve, with the rising of temperature, the value of "g_m" in the equation of B.E.T. (3) and the value of "g'_m" of water-content showing the value of "a", corresponding to point "R" on curve III, became less. (But in general, the values of "a" corresponding to point "R" are comparatively independent from the change of temperature.) Therefore, the water-content, "g_P" estimated at point "P" on the drying curve of 40°C (Curve IV) seems to be somewhat larger than those of "g'_m" (and "g_m") on the "g—a" curve estimated at 40°C. From results as above obtained, the turning point "P" on the drying curve of 40°C is to be regarded as the changing point from the first stage of falling rate of drying to the second stage. The value of "a" of the water activity of sample showing the water-content, "g_P", is below about 0.7. According to Bull's result[22)] and the present author's results obtained for gelatine, fish meat protein and raw fish meat previously described in Paper II[1b)], water of which the value of "a" is below 0.7, is to be regarded as the so called molecular theoretical Bound Water which mainly consists of hydrogen binding. Therefore, in such a range of values of "a" below 0.7, the rate of drying remarkably decreased and the second stage of falling rate of drying seems to be in progress.

The amount of Bound Water obtained by the cobaltous chloride method (Oyagi's method) (that is the value of the amount of water-content at points S, T, on curves I and IV) is far larger than that of water-content at "g_P" or "g'_m" in each Fig. Further, as the value of "a" corresponding to the amount of Bound Water, "$g_{B.P.}$", is in the range of 0.75~0.95 as above stated, this Bound Water should be regarded as colloidal Bound Water rather than as molecular theoretical Bound Water. In this experiment, as to the above consideration, there seemed to be no particular difference among the kinds of fish meat.

3. Conclusion

In the case of drying of fish meat at 40°C, the absolute amount of water-content (gm of water per gm of dried matter) "g", decreased in the initial period of drying with the drying time "t" almost in the linear relation. For example, when the value of "g_P" was 0.218 for crucian carp meat, and the values of "g_P" were 0.14~0.22 for other fish meat, the drying coefficient "λ_2" decreased rapidly to 10~18% of the initial drying coefficient "λ_1". The value of "a" in each sample

Table 30. The influence of Bound Water upon the velocity of drying of fish meat

Sample meat	Raw fish meat			Water-content at point of "B.P."	Water-content at point of "P"	$\frac{g_P}{g_{B.P.}} \times 100$ (%)	Water-content being in a state of equilibrium in the drying at 40°C (%)
	R.T.* (%)	R.B.* (%)	R.F.* (%)	A($g_{B.P.}$) (% in original)	B(g_P) (% in original)		
Crucian carp	81.6	10.6	71.0	36.6	17.9	48.9	13.2
"Soi"	79.9	9.0	70.9	30.9	17.4	56.3	16.0
Yellow-tail	75.6	12.2	63.4	33.3	16.0	48.1	13.7
Atka-mackerel	80.0	8.8	71.2	30.6	16.3	53.3	13.1
Sardine	76.8	14.1	62.7	37.7	9.9	26.3	8.7
Mackerel	74.3	14.3	60.0	35.8	17.7	49.5	14.9
Tuna	71.8	17.8	53.0	38.7	14.5	37.5	9.7
"Umitanago"	78.0	—	—	—	16.0	—	12.0
"Hirame"	78.9	11.4	67.5	35.0	13.0	37.2	11.8
"Magarei"	81.4	8.9	72.5	32.3	14.5	44.9	12.3
Squid	74.6	18.5	56.1	42.1	12.3	29.2	9.1
"Botan-ebi"	78.0	12.6	65.4	36.4	16.0	44.0	13.9

* R.T.=Total Water content in raw material (%).
R.B.=Bound Water content in raw material (%).
R.F.=Free Water content in raw material (%).

corresponding to each amount of water-content, "g_P" as above stated, were 0.40~0.65 for various kinds of fish meat.

As the values of the constant "g_m" in B.E.T. equation (3), (an amount of water required to cover the surface of a molecule of fish meat protein with a monomolecular water layer), 0.07~0.15 were obtained. This is almost one half of the amount of water-content at "g_P".

According to Bull[22] and the present author[1b], water having the amount of water-content of twice the value of "g_m", or water having below 0.7 of the water-activity "a" is considered to be molecular theoretical Bound Water. Therefore, in the case of drying of fish meat at 40°C, the velocity of drying of the meat decreased by the existence of molecular theoretical Bound Water. In the 2nd, 3rd and 4th columns of Table 30, the total amount of water-content of fresh raw fish meat (R.T. %), the amount of Bound Water (R.B. %), and that of Free Water (R.F. %) are shown respectively. In column 5, the percentage of the amount of water-content regarded as only Bound Water (A%) is shown. In column 6 the amount of water-content at the turning point "P" of the drying curve at 40°C (B%) is shown.

As seen in Table 30, the total water-content of fresh raw fish meat for crucian carp was 81.6%. In this total amount of water-content, there are included 10.6% of Bound Water and 71.0% of Free Water. When this fish meat was dried, the total water-content decreased to 36.6% (A); the water in this dried fish meat is to be regarded as colloidal Bound Water. With the progress of drying, when the total amount of water-content reduced to 17.9% (B), the velocity of drying at 40°C decreased rapidly, and the water in sample at that time went into the sphere of molecular theoretical Bound Water.

The ratio of B to A ($B/A \times 100$) was 48.9% for crucian carp meat as shown in column 7 of Table 30, and about 37~57% for other kinds of fish excepting 26.3% for sardine and 29.2% for squid. That is to say, the velocity of drying decreased rapidly in the range of 37~57% to the amount of Bound Water estimated by cobaltous chloride method. However, in the drying at 40°C, the amount of water-content being in a state of equilibrium was 13.2% for crucian carp, and 9~16% for other kinds of fish meat. In sun-drying of fish meat, the limit total amount of water-content of drying is considered generally to be 18~25%. In such values the velocity of drying may be remarkably influenced by the presence of colloidal Bound Water, and drying time is considered to become longer.

IV. ON THE RELATION BETWEEN THE "DRIP" FORMATION AND THE AMOUNT OF BOUND WATER DURING THE REFRIGERATION OF FISH MEAT

"Drip" is the liquid separated from the frozen fish or meat after being thawed. There are two kinds of "Drip". One of them is "Free drip" which is separatable naturally or by light pressure. The other is "Expressible drip" which separated from the frozen fish or meat by heavy pressure, *e.g.* 1~2 atmosphers,

gauge pressure). Those "Drips" contain a large amount of various nutritive chemical components and tasty or flavourous matters. The larger the amount of those separated liquids becomes, therefore, the lower becomes the commercial value of the frozen goods.

Many studies on the formation of "Drip" have been made up to the present[30]. According to results obtained, the principal cause of the formation is the chemical and physical changes, *e.g.* the denaturation of meat protein and the destruction of muscle tissue by the formation of ice crystals when the material is refrigerated. In the present experiment, the author has studied the relation between the formation of "Drip" and the denaturation of fish meat protein during storage (at −18°C) of the fish meat quick-frozen at −30°C, in order, as nearly as possible, to prevent the destruction of muscle tissue by keeping them at very low temperature. Then he studied the change of properties of Buond Water owing to the denaturation of fish meat protein by formation of ice crystals, (that means the dehydration or the condensation of electrolytes in fish meat), during the refrigeration and storage, by means of comparing the relation between the water-content and the relative vapour pressure (water-activity).

Next, a study was made of the change of the hydrating affinity of the "Drip" only, and of fish meat after separation of "Drip" respectively.

To prevent the formation of "Drip", Tarr[31] has studied the soaking of fish meat in NaCl soluiton before refrigeration.

The present author has studied the hydration of fish meat protein by various kinds of salt in order to learn the way to prevent the formation of "Drip".

1. Experimental part

(1) *On the relation between the freshness of the fish meat and the amount of "Drip" formed*

(i) Sample

As sample, fillet meat of Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ) was employed.

(ii) Estimation of the amount of "Drip"

The estimation of the amount of "Drip" was carried out continuously for the "Free drip" and then "Expressible drip".

(A) Estimation of "Free drip"

About 200 gm of fillet meat sliced from the frozen fish was hung in the room (room temperature, 16°~19°C; relative humidity, about 75%) and thawed. The liquid which separated from the fillet meat during the thawing was received into a vessel.

The proportion (%) of the decreasing weight to the initial weight of the fillet meat used was expressed as the amount of "Free drip". The time when the dripping was finished over is the limit time for the estimation of "Free drip". It required about 16 hours to complete the estimation. During that time, the evaporation of moisture from the surface of the fillet meat was out of consideration. The samples were employed in as nearly the same shape and same size as possible.

(B) Estimation of "Expressible drip"

The sample from which "Free drip" was separated out sufficiently was put into a canvas bag, and this bag was placed in a rectangular gunmetal box (the size of the mouth was 7 × 7 cm) as shown in Fig 21.

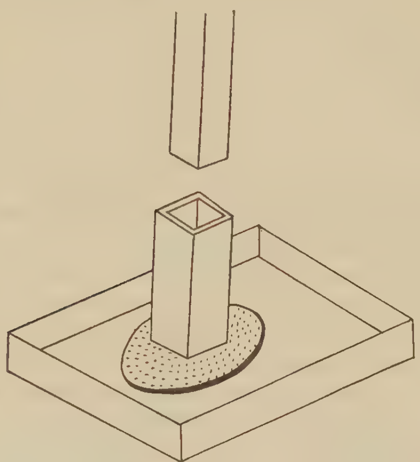


Fig. 21. Estimating apparatus of the amount of "Drip" in frozen fish muscle

Then the bag was pressed by a pressing machine. The pressure was estimated by a pressure gauge. The liquid separated from the sample was received in a vessel. The canvas bag was weakly pressed during the initial period; then after 10 minutes strongly pressed at 0.5 kg/cm^2 of power. The pressure was continued in the same strength during 20～30 minutes. The liquid thus separated is obtained as "Expressible drip". The amount of "Expressible drip" is expressed by the proportion (%) of the amount of the liquid separated to the initial weight of the fillet meat taken before the "Free drip" estimation was carried out.

(iii) Experimental method

Five raw Atka mackerel, of which the freshness was very good, in the period of rigor mortis were brought to the laboratory. One of them was employed as a raw sample (Sample E) without refrigeration. This sample was immediately used for the estimation of the amount of "Drip". The other sample was immediately frozen in an Iwamoto's contact-plate freezing apparatus at −30°C for 14 hours (Sample A) and stored at −18°C for 25 days. This sample was used for the estimation of the amount of "Drip".

Each of the three other Atka mackerel were left alone in a room (room temperature, 20°C±3°C) and then were frozen an intervals of 4 hours leaving in the same apparatus. This leaving in a room was done in order to decrease the freshness in various stages. Those samples were called B, C, and D, respectively from the different leaving times.

Sampl D left alone for 12 hours was unfresh; the meat became soft but edible. The amount of volatile basic nitrogen estimated for Sample E (fresh

raw without refrigeration) was 2.5 mg%; for Sample D it was 20 mg%. The freshness of this Sample D seemed to be in a stage before incipient putrefaction.

(iv) Experimental results and discussion

The results obtained were shown in Table 31 and Fig. 22.

From Table 31 and Fig. 22, the total amount of "Drip", including both categories, "Free" and "Expressible", is seen to increase with the falling of the freshness of samples used.

As seen in the proportion of "Free drip" or "Expressible drip" to the "Total drip", the values of "Free drip" increased, and those of "Expressible drip" decreased with the falling of freshness of samples.

According to the results obtained in the previous Section II, with the decomposition of fish meat protein, the proportion of the amount of Bound Water to Total water-content decreased, and that of Free Water relatively increased; further, the electrical resistance decreased (That is, there was an increasing of electric conductivity). The author has inferred from the results above stated

Table 31. Difference of the amount of "Drip" from the frozen fish meat of different stages of freshness (Atka mackerel meat frozen at −30°C and stored at −18°C for 25 days)

Sample	Free drip (F.D.)		Exp. drip (E.D.)		Total drip* (T.D.) (%)
	F.D. (%)	$\frac{F.D.}{T.D.} \times 100$ (%)	E.D. (%)	$\frac{E.D.}{T.D.} \times 100$ (%)	
A	7.6	39.5	11.6	60.5	19.2
B	9.8	45.0	12.0	55.0	21.8
C	11.1	47.6	12.2	52.4	23.3
D	15.0	51.0	14.4	49.0	29.4
E**	0	0	2.0	100	2.0

* T.D.=F.D.+E.D.

** Unfrozen meat

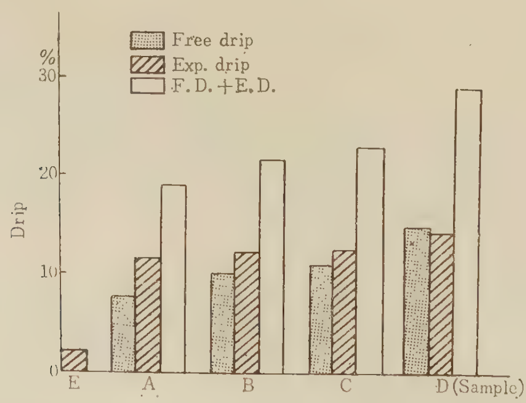


Fig. 22. Relation between the amount of "Drip" and the freshness of fish meat (Atka mackerel)

that the binding affinity of water in fish meat decreases with the decomposition of fish meat protein.

It is easy to infer, therefore, that the amount of "Free drip" estimated may increase when unfresh meat is frozen.

It is important to use the raw fish meat as fresh as possible in order to minimize the amount of formation of "Drip". In the control-sample (Sample E) which was raw without refrigeration, the leakage of "Free drip" could not found, and the amount of "Expressible drip" was estimated at only about 2% by weight as shown in Table 31.

On the contrary, in Sample A frozen under very fresh state, the amount of "Free drip" was 7.6%, "Expressible drip" was 11.16%.

According to the experimental results of the Korean Fisheries Experimental Station[32], the amount of "Drip" of fresh frozen fish meat differs with the kind of fish, and thawing temperature; the amount of "Expressible drip" was obtained to be 5~45% in the range of 5°~45°C of experimental temperature.

From those results, it may be generalized that the formation of "Drip" is unpreventable in the frozen fish meat, and the formed amount of "Drip" increases with longer time of freezing storage. Therefore, it is clear that the formation of "Drip" is remarkably influenced by the properties of fish meat, freezing conditions and storage conditions after refrigeration. In the further experiments, the fish meat having similar freshness was employed in order to equalize the internal factors before the refrigeration.

(2) *The relation between the amount of "Drip" formed and the storage period of frozen fish meat*

In this experiment, ten Atka mackerel were quickly frozen at −30°C by Iwamoto's quick freezing apparatus, and stored at −17°C~−18°C for 2 months. At certain definite intervals each one fish was taken and the amounts of "Free drip" and "Expressible drip" were estimated by the method previously described.

The results obtained are shown in Table 32 and Fig. 23.

As seen in Fig. 23 the amount of "Free drip" increased clearly with the longer time of storage. On the contrary, the amount of "Expressible drip" was

Table 32. Change of the amount of "Drip" during the storage of frozen Atka mackerel meat (Freezing temperature −30°C, freezing time 14 hrs, storage temp. −17°~−18°C)

Drip \ Days	2	5	8	10	16	23	26	38	57
Free drip (%)	5.6	4.5	7.0	9.4	10.6	12.2	10.9	15.1	17.6
$\frac{\text{F.D.}}{\text{E.D.}+\text{F.D.}}\times 100$	(31.8)	(17.4)	—	(41.5)	(46)	(46)	(46.4)	(57.5)	(55)
Exp. drip (%)	12.0	21.0	—	16.9	12.5	14.4	12.6	12.2	14.4
$\frac{\text{E.D.}}{\text{E.D.}+\text{F.D.}}\times 100$	(68.2)	(86.2)	—	(58.5)	(54)	(54)	(53.6)	(42.5)	(45)
E.D.+F.D. (%)	17.6	25.5	—	22.6	23.1	26.6	23.5	27.3	32

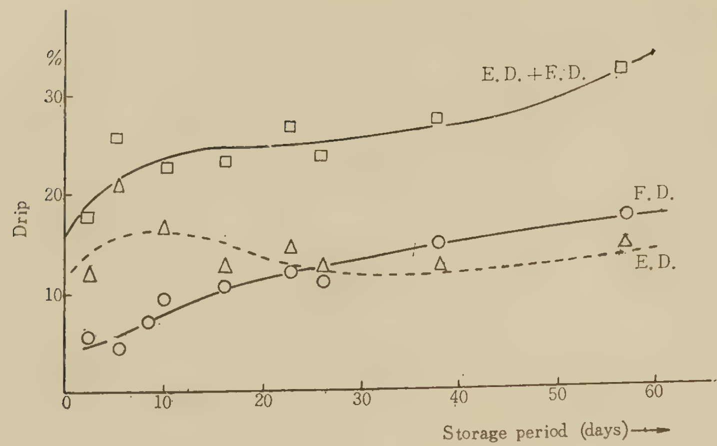


Fig. 23. Change of the amount of "Drip" during the storage of frozen Atka mackerel meat

larger than that of "Free drip" in the initial period of storage after the freezing, but the former amount decreased with the longer time of storage, and after 25 days' storage the amount became little.

The sum of "Expressible drip" and "Free drip" (the total amount of "Drip") increased clearly with the longer time of storage just as amount of "Free drip" did; this agrees with the results of Moran[33]. As seen in Table 32, the ratio of "Free drip" to the "Total drip" increased with the longer time of storage; and, contrariwise, the ratio of "Expressible drip" decreased. This seems to be because a part of "Expressible drip" which has been estimated in the initial period of the storage changed to "Free drip" with the passage of the storage time. It has generally been said that from fish meat which was slowly frozen a larger amount of "Free drip" separates than that of "Expressible drip"; on the contrary, from quickly frozen fish meat the amount of "Expressible drip" has been said to exceed that of "Free drip". However, according to the present author's results which were obtained from fish meat quickly frozen at −30°C, the amount of "Expressible drip" was more than that of "Free drip" only in the initial period of storage, thereafter, the amount of "Free drip" becomes more than that of "Expressible drip".

When as a cause of the formation of "Drip", histological change of fish meat tissue at the freezing is considered, the size of ice crystals formed in slowly frozen fish meat is large; the destruction of muscle tissue, therefore, is considered to be great and the amount of "Free drip" is much. On the other hand, the size of ice crystals formed in quickly frozen fish meat is small, therefore the destruction of muscle tissue is considered to be slight, and the amount of "Expressible drip" is little. However, even in the quickly frozen meat where the destruction of tissue is considered to be slight, the amount of "Free drip" increased with the longer time of storage, even at a low temperature such as −18°C. Hence the formation of "Drip" in this experiment is considered to be mainly due to the

dehydrating denaturation of fish meat protein rather than to the histological damage.

(3) *The comparison of the curve of the water-content and the water-activity of sample, "g—a" in the fresh raw meat and frozen meat*

Fresh raw meat of Atka mackerel and the same meat which was frozen quickly at −27°C and stored at −18°C for two weeks were employed for the vapour pressure measurement at 15°C, then the relation between the water-content, "*g*" (gm of water per gm of dried matter) and the relative vapour

Table 33. Relation between the water-content "*g*" and the water-activity "*a*" in fresh raw and frozen Atka mackerel meat

Fresh raw meat		Frozen meat	
Water-content "*g*"	Water-activity "*a*" or "p/p_0"	"*g*"	"*a*" or "p/p_0"
0.774	0.91	1.16	0.91
0.568	0.87	0.38	0.79
0.344	0.83	0.16	0.47
0.223	0.63	0.13	0.26
0.213	0.48	0.11	0.19
0.210	0.41	0.09	0.10
0.150	0.14	—	—
0.110	0.06	—	—

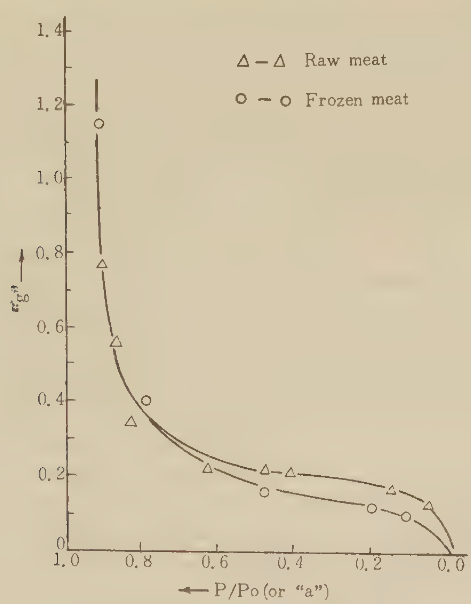


Fig. 24. "*g—a*" curves for the fresh raw and frozen Atka mackerel meat

pressure, p/p_0, (or water-activity "*a*") was observed.

The estimation of vapour pressure was carried out with the same apparatus as described in the previous Paper I[1a]. The equivalent pressure between the vapour pressure of pure water and that of water in the sample was estimated. The results obtained are shown in Table 33 and Fig. 24.

As seen clearly in Fig. 24, the relation between "*a*" and "*g*" is expressed by almost the same curve in the fresh raw fish meat and frozen meat until the value of "*a*" is 1.0~0.6 and the value of "*g*" is 0.2, but when the value of "*g*" is below 0.2, the value of "*a*" of the frozen meat is higher than that of fresh raw meat in comparison with the same water content, "*g*". That is to say, the curve "*g—a*" of the former slips down to the left side of that of the latter. This fact shows clearly that the differential molal free energy of water in the frozen fish meat decreases to a less degree than that of water in the fresh raw fish meat.

Here, when the differential molal free energy of pure water is $F°$, and that of water in the sample at the same temperature is $\bar{F}$, the difference of the two is shown as following equation (8) as stated in previous Paper II[1b].

$$\Delta\bar{F}=F^{\circ}-\bar{F}=-RT\ln p/p_0 \qquad (8)$$

In equation (8), when the values of $\Delta\bar{F}$ were calculated and compared for each amount of water-content, "*g*", results were otained as set forth in Table 34 and Fig. 25.

As seen clearly in Fig. 25 the value of $\Delta\bar{F}$ of water in the frozen fish meat is less than that in the fresh raw fish meat when the water-content "*g*" is below 0.3.

That is to say, the differential molal free energy of water in frozen fish meat is shown to come up to that of pure water, in other words, water in frozen fish meat became free state. In Fig. 24, water of which the value of "*a*" is below 0.7 is considered to be molecular theoretical Bound Water, therefore in this case, it is considered that the formation of "Drip" from the frozen meat is due to the loss of hydration of fish meat protein rather than due to a physical factor such as the destruction of tissue by means of freezing of fish muscle.

Table 34. The comparison of the differential molal free energy of water in fresh raw and in frozen Atka mackerel meat

Water-content "*g*"	Fresh raw meat		Frozen meat	
	"*a*" or "p/p_0"	$\Delta\bar{F}$ (cal/mol)	"*a*" or "p/p_0"	$\Delta\bar{F}$ (cal/mol)
1.0	0.92	47.8	0.92	47.8
0.7	0.90	60.5	0.90	60.5
0.4	0.82	114	0.82	114
0.3	0.73	180	0.73	180
0.2	0.40	525	0.54	353
0.15	0.14	1,100	0.36	586
0.10	0.05	1,720	0.13	1,170

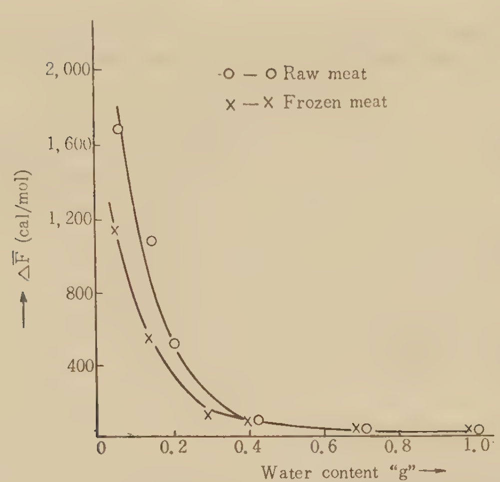


Fig. 25. "$g-\Delta\bar{F}$" curve for the fresh raw and frozen Atka mackerel meat

(4) *The comparison of curves "g—a" of the frozen fish meat, fish meat dripped out and "Drip" liquid respectively*

Comparisons were made of the cruves "*g—a*" of Atka mackerel meat which was quickly frozen at −30°C and stored at −18°C for two weeks, of the meat from which "Free drip" had been lost and of the meat from which "Expressible drip" had been removed. The results obtained are shown in Table 35 and Fig. 26.

In Fig. 26, curve I is the curve "*g—a*" of frozen fish meat, curve II shows that for the fish meat less "Free drip", curve III shows that freed of "Expressible drip", curves IV and V show those for "Free drip" only and "Expressible drip" only, respectively. As seen clearly in those curves, the values of the water-activity "*a*" in curves II and III became smaller than that in Curve I from the point of about 0.65 of the value of the water-activity "*a*" (that is 0.3 of the water-content "*g*"), when those curves were compared at the same amount of water-content. That is to say, curves II and III slipped above curve I at below 0.65 of the value of the water-activty, "*a*".

This fact shows that the differences in the differential molal free energy of pure water and that of water in "Drip"-freed fish meat were greater than those of frozen fish meat. Water in fish meat from which "Drip" had been separated seems to have comparatively stronger affinity with the fish meat protein.

When the water-activity "*a*" is above 0.65, curves I, II and III were in piles and became one curve just as seen in Fig. 26.

There is some difference between curves II and III, but curve III seemed to slip down to the right side. Therefore, water in fish meat from which "Expressible drip" was separated has stronger affinity with the fish meat protein than was found in fish meat from which "Free drip" was separated.

From the fact that the values of "*a*" on curve V slipped down to the right side on curve IV, the "Expressible drip" (as curve V) seems to show properties of water having large differential molal free energy in comparison with "Free

Table 35. The relation of curves "$g-a$" among the frozen Atka mackerel meat, the meat from which "Drip" had been separated and "Drip" liquid only

Sample	Water-content "g"	Water-activity "a" or "p/p_0"
Frozen meat (I)	0.77 0.27 0.22 0.19 0.18	0.83 0.63 0.38 0.37 0.24
Meat dripped out Free drip (II)	0.98 0.34 0.25 0.25 0.24	0.85 0.72 0.49 0.46 0.33
Meat dripped out Exp. drip (III)	0.65 0.26 0.24 0.22	0.81 0.54 0.42 0.16
Free drip (IV)	6.17 0.64 0.20 0.13	0.87 0.62 0.52 0.32
Exp. drip (V)	3.76 0.47 0.28 0.22	0.76 0.67 0.10 0.05

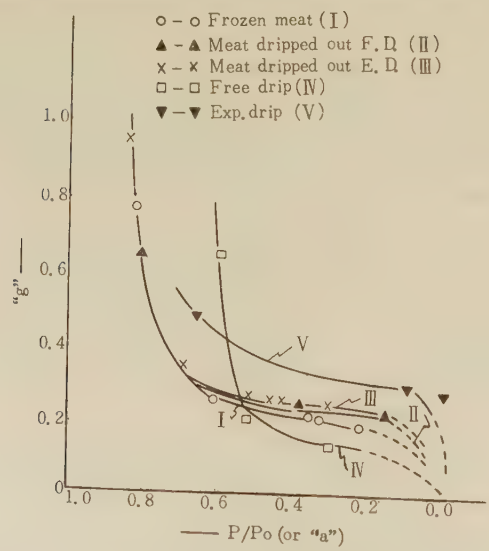


Fig. 26. "$g-a$" curves for frozen Atka mackerel meat, the meat from which "Drip" had been separated and "Drip" liquid only

drip", but it is impossible to affirm positively the result as above stated, because there is some impurity (meat pieces *etc.*) in both types of "drip".

From article (1)～(4), a principal cause of the formation of "Drip" in quickly frozen fish meat during storage may be stated to be the decrease of affinity of Bound Water with fish meat protein owing to the denaturation of fish meat protein, because the value of differential molal free energy of water regarded as molecular theoretcal Bound Water of whch the value of water-activity "*a*" is below 0.7 on the curve "*g—a*", decreased during freezing storage.

(5) *Examination of soaking of fish meat in various kinds of salt solution as a method for preventing the formation of "Drip"*

As previously stated, Tarr[31] has devised a method of soaking fish meat in NaCl solution for a very short time before freezing in order to prevent the formation of "Drip".

The present author has examined this soaking method. In previous Experiments (1)～(4), a cause of the formation of "Drip" during freezing storage was considered to be that water in fish meat is set free by the dehydrating denaturation of fish meat protein, and then the value of $\Delta\bar{F}$ of water in the fish meat decreases. From the fact as above obtained, if the method of increasing the value of $\Delta\bar{F}$ has been found for raw fish meat, it would seem possible to prevent to a certain degree the formation of "Drip" during freezing storage. The soaking of fish meat into salt solution works to decrease the vapour pressure of water in the surface of fish meat, that is to say, it means the increase of $\Delta\bar{F}$. The effect of the soaking method which devised by Tarr[31] is due to the increase of $\Delta\bar{F}$. According to Tarr, when an adequate concentration of NaCl solution having high swelling activity for fish meat was employed, the amount of "Drip" became much less. Taking those results into consideration, the soaking of fish meat in solution having an adequate values of pH seems to have the same preventing effect for "Drip" formation as the soaking in salt solution. As a matter of fact, Taylor[34] has studied a method of preventing the formation of "Drip" by soaking in solution having certain definite values of pH. According to his results the amount of "Drip" decreased with the increasing of the value of pH. The present author has studied the influence of hydrogen ion concentration and kinds of salt of the soaking soluiton upon the swelling of fish meat.

(i) The influence of hydrogen ion concentration of soaking solution upon the swelling of fish meat

As samples, Atka mackerel meats cut in pieces of about $3 \times 3 \times 2$ cm size (15～20 gm in weight) were used. Each piece was put into 10 times volume of buffer solution having various values of pH, and left alone in the room for 24 hours. After soaking, the weight of each sample was estimated. The degree of the swelling "*S*" was expressed by the proportion of the weight of soaked sample, "*W*", to that of sample before soaking, "W_0", (W/W_0, %).

The result is shown in Table 36 and Fig. 27.

As seen clearly in Fig. 27, the degree of swelling of fish meat is the smallest at pH 6～7, and it increased at pH values of above or below 7. Generally, it is said that ampholyte such as protein containing diamino acid and dicarboxylic acid indicates the maximum degree of the swelling at pH 2～3 and pH 10～12[35].

Table 36. The degree of swelling of Atka mackerel meat soaked in buffer solutions having various pH values

pH	2.25	4.05	6.12	7.01	8.02	9.34	10.84
S	1.19	1.13	1.13	1.11	1.17	1.16	1.24

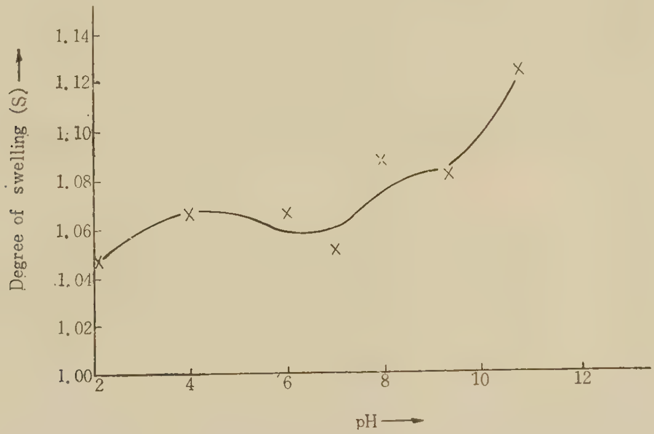


Fig. 27. Relation between the value of pH and the degree of swelling of Atka mackerel meat

Curve "*g*—*a*" was obtained for samples which were soaked in solution having 4.05, 7.01 and 10.84 pH values as shown in Table 37 and Fig. 28.

In Fig. 28, curve I shows the relation between "*g*" and "*a*" for fresh raw fish meat without soaking (as control sample), curve II shows that for fish meat soaked in pH 4.05 solution, curve III shows that in pH 7.01 solution, and curve IV shows that in pH 10.84 solution.

As seen in Fig. 28, the values of "*a*" in curves II, III and IV were smaller than "*a*" in curve I at the same amount of water-content, and those curves slipped down to right side from curve I. That is, by soaking of fish meat in

Table 37. Relation of "*g*" and "*a*" of Atka mackerel meat soaked in solutions having various pH values

Fresh raw meat		Soaked meat in pH 4.05 soln.		Soaked meat in pH 7.01 soln.		Soaked meat in pH 10.84 soln.	
"*g*"	"*a*"	"*g*"	"*a*"	"*g*"	"*a*"	"*g*"	"*a*"
0.77	0.91	1.40	0.93	1.80	0.87	1.24	0.89
0.57	0.87	0.41	0.82	0.54	0.80	0.35	0.81
0.34	0.83	0.27	0.56	0.23	0.61	0.23	0.53
0.22	0.63	0.19	0.06	0.17	0.10	0.16	0.23
0.21	0.41	0.15	0.04	0.15	0.06	0.13	0.13

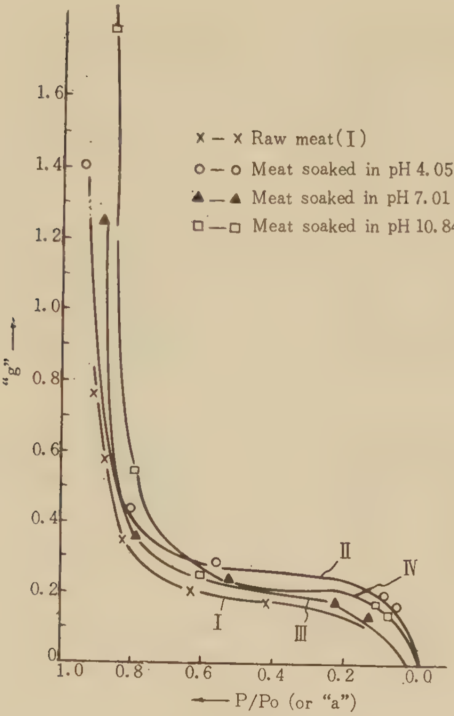


Fig. 28. "$g-a$" curves for Atka mackerel meat soaked in solutions having various pH values

buffer solution the degree of swelling increased and the value of $\Delta\overline{F}$ of water in the fish meat increased.

When those soaked fish meat samples are frozen, with the decrease of the value of $\Delta\overline{F}$ of water in fish meat during the storage, the formation of "Drip" may be somewhat lessened as suggested in above article. In practical case of soaking of fish meat in buffer solution, it is feared that the soaking is rather effective in the surface part of fish meat, but it may not be so effective in the inner part of fish meat, because the soaking solution does not penetrate perfectly into the inner part of fish meat in which the water has the same property as that of fresh raw fish meat even after the soaking. However, even if water in the inner part of fish meat should be set free during the freezing storage, as there is a water layer having large value of $\Delta\overline{F}$ and having hydration in the surface part of the fish meat, the amount of "Drip" which may be separated when thawed would seem to be slight.

(ii) The relation between the concentration of NaCl solution and the value of pH which exerted influence upon the degree of swelling of fish meat

Table 38 and Fig. 29 show the degree of swelling of Atka mackerel meat during the soaking of it in the NaCl solution having various molar concentration for 24 and 48 hours. In the last column of Table 38, the values of pH after soaking are tabulated.

Table 38. Relation between the concentration of NaCl solution and degree of swelling of Atka mackerel meat

Conc. of NaCl (Mol)	Degree of swelling, "*S*"		pH (after soaking)
	24 hrs. after	48 hrs. after	
0 (Dist. water)	1.00	—	—
0.05	1.23	1.19	6.16
0.1	1.20	1.18	6.22
0.2	1.17	1.19	6.14
0.5	1.21	1.24	6.12
1	1.18	1.24	5.93
2	1.09	1.15	5.85
3	1.10	1.20	5.75
4	0.98	1.06	5.73
5	0.94	0.96	5.65
6	0.92	0.96	5.64

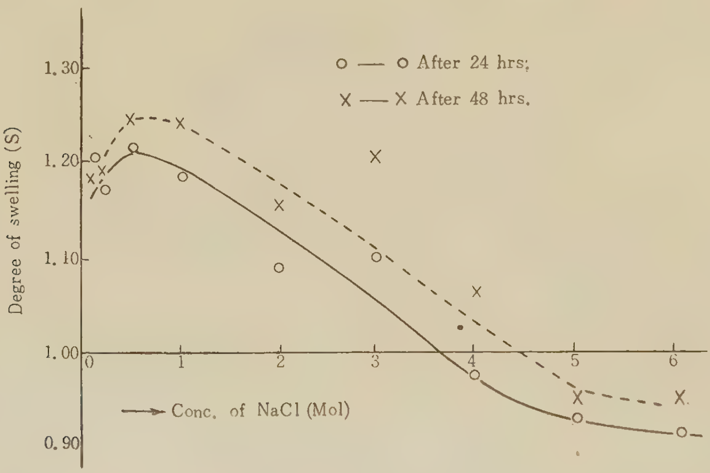


Fig. 29. "Conc.—*S*" curve for Atka mackerel meat in NaCl solution

As seen in Fig. 29, after 24 hours' soaking, the degree of swelling of fish meat showed the maximum value in about 0.5 Mol (2.9%) of NaCl solution. Below 0.5 Mol or above 1 Mol (5.8%) the degree of swelling decreased, and above 3.5 Mol (20.5%) the value of the degree of swelling fell below 1. After 48 hours' soaking, the values of degree of swelling are generally larger than that those of 24 hours' soaking. The maximum value of degree of swelling was shown in 0.8 Mol (4.68%) of NaCl solution, and above 4.5 Mol (26.3%) its value of swelling dropped below 1.

According to Tarr's statement[31], the relation between the degree of swelling of halibut meat (5 × 5 × 2.5 cm in size) and soaking time was attained to equilibrium after 5~6 days in 2% NaCl solution.

In his result, the maximum value of degree of swelling was shown in 5~7% (0.85~1.2 Mol) of NaCl solution.

The present author's result almost agreed with Tarr's result.

As seen in Table 38, the values of pH of various concentrations of NaCl solution after soaking of fish meat varied to the acidic side with the increase of the concentration of NaCl solution.

As to the swelling of fish meat in salt solution, there is perhaps some relation between the concentration of salts and the value of pH of that salt solution.

The present author has estimated the degree of swelling of Atka mackerel meat after soaking in 0.86 Mol (5%) NaCl solution of which the values of pH varied 2~11. The value of pH of the NaCl solution was adjusted by addition of 1 N H_2SO_4 or 1 N NaOH solution. The results obtained are shown in Table 39 and Fig. 30.

Table 39. The degree of swelling of Atka mackerel meat after soaking in 0.86 Mol (5%) NaCl solution of which the value of pH varied 2~11

pH	2.35	4.35	6.14	6.96	8.15	9.13	10.85
S	1.32	1.30	1.37	1.34	1.26	1.33	1.26

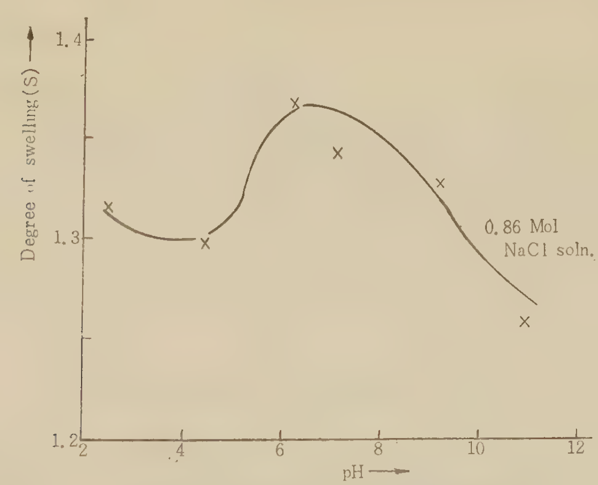


Fig. 30. "pH—*S*" curve for Atka mackerel meat in 0.86 Mol (5%) NaCl solution

As seen in Fig. 30, the degree of swelling of fish meat "*S*", is the minimum at about pH 4, it increased rapidly at pH 4~6, it is the maximum at about pH 6~7, and it decreased above pH 7~11. Below pH 4 the value of "*S*" increased somewhat.

Comparing Fig. 30 with Fig. 27, the swelling in acidic side is restrained below pH 5 by the adding of salts, and contrariwise is promoted above pH 5. Those results agreed with Tarr's findings.

The experiment shown in Fig. 27 was carried on by using of buffer solution,

that in Fig. 30 was carried on by adding of H_2SO_4 or NaOH solution. Comparing the values of "*S*" in both experiments, the value of "*S*" in Fig. 30 is seen to be larger than "*S*" in Fig. 27.

In Tarr's studies, both experiments were carried on by using of the same buffer solution. According to Tarr, in the range of pH 2~4 of the acidic side the value of "*S*" which was added with salt was larger than "*S*" without adding of salt, and in the range of pH 5~7, the result was contrary.

From the result as above obtained in case of solution to which was added no salt, following conclusions can be drawn.

The maximum swelling was showed at pH 2 or pH 10, and the concentration of NaCl solution for the maximum swelling was 0.5~1.0 Mol (2.93~5.8%). In the case of adding of salts, the pH value of maximum swelling changed to pH 6~7 from pH 10. In acidic side, the degree of swelling decreased more than in the case of no adding of salt.

From those findings, the soaking of fish meat in solutions of pH 2 or 10 is considered to be effective to prevent the formation of "Drip", but this soaking is not practical. Particularly in the acidic side, the denaturation of fish meat protein occurs remarkably during the freezing storage[36], therefore the soaking of fish meat in solution of pH 2 shows least effect.

To prevent the formation of "Drip", the soaking of fish meat in NaCl solution of which the concentration is 0.85 Mol (5%) NaCl solution with pH value of 6~7 seems to be the best for practical use.

Concerning practical experiment, according to Tarr's result, when the halibut meat was soaked in the solution (pH 7.10, 5% NaCl) containing 2.5 gm of NaCl and 50 cc of 0.2 N NaOH, and the soaked fish meat left alone at 1°~5°C for 24 hours, the amount of "Expressible drip" was 12%. In the case of soaking in 5% NaCl solution, the amount of it was 18.8%; on the other hand, in the case of the use of dil. HCl solution or water only, the amount of it was 63~52%. The present author's result above obtained agreed with Tarr's result. That is to say, it is the best to use for the soaking solution 5% (0.85 Mol) NaCl solution with pH 6~7 value.

(iii) The influence of various kinds of salt upon the degree of swelling of fish meat

Following salts and chlorides were used as substances for addition:

Na-salts —NaCl, Na_2SO_4, $NaNO_3$, $Na_2B_4O_7 \cdot 10aq.$, $Na_3PO_4 \cdot 12aq.$
CH_3COONa, $C_3H_4(OH)(COONa)_3 \cdot 3aq.$

K-salts —KI, KCl, KNO_3, K_2SO_4, K_2CO_3, CH_3COOK

Chlorides—KCl, $CaCl_2$, $MgCl_2 \cdot 6aq.$, NH_4Cl, NaCl

A 0.6 Mol soaking solution was prepared with those salts and chlorides, and Atka mackerel meat was soaked in each solution for 24 hours. The values of degree of swelling were estimated as above described. The results obtained are shown in Table 40 and Fig. 31.

From Fig. 31, when cation is the same and anion is different, following facts were observed;

In Na-salts: the degree of swelling "*S*" decreased in the following order:

Table 40. The degree of swelling of Atka mackerel meat by the various salts (0.6 Mol soln.)

Salts	"S"	Salts	"S"
NaCl	1.25	K_2SO_4	0.91
Na_2SO_4	0.98	K_2CO_3	1.29
$Na_2B_4O_7 \cdot 10aq.$	1.06	CH_3COOK	1.06
$Na_3PO_4 \cdot 12aq.$	0.98	KCl	1.37
CH_3COONa	1.43	$CaCl_2$	1.15
$NaNO_3$	1.33	$MgCl_2 \cdot 6aq.$	1.20
$C_3H_4(OH)(COONa)_3 \cdot 3aq.$	0.85	NH_4Cl	1.24
KI	1.39		
KNO_3	1.35		

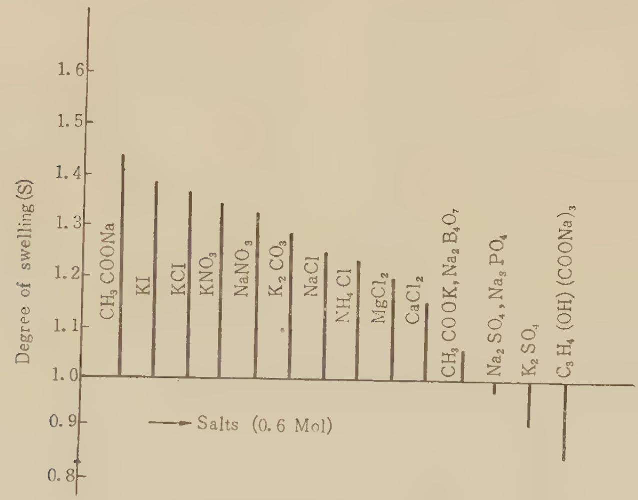


Fig. 31. The degree of swelling of Atka mackerel meat by the various salts (0.6 Mol soln.)

$$CH_3COO^- > NO_3^- > Cl^- > B_4O_7^{--} > SO_4^{--} > PO_4^{---} > C_3H_4(OH)\cdot(COO)_3^{---}$$

The values of "S" of Na_2SO_4, Na_3PO_4 and $C_3H_4(OH)(COONa)_3$ were less than 1.

In K-salts, the degree of swelling "S" decreased in the following order:

$$I^- > Cl^- > NO_3^- > CO_3^{--} > CH_3COO^- > SO_4^{--}$$

The value of "S" of K_2SO_4 is less than 1.

When anion is the same and cation is different, the values of "S" decreased in the following order:

$$K^+ > Na^+ > NH_4^+ > Mg^{++} > Ca^{++}$$

The order of decreasing of the values of "S" of various salts and chlorides is as follows:

$CH_3COONa > KI > KCl > KNO_3 > NaNO_3 > K_2CO_3 > NaCl > NH_4Cl >$
$MgCl_2 > CaCl_2 > CH_3COOK > Na_2B_4O_7 > Na_2SO_4 > Na_3PO_4 > K_2SO_4 >$
$C_3H_4(OH)(COONa)_3$

The order of decreasing of the values of "S" is different with ionic strength. Those results obtained by the present author were in the case of the use of 0.6 Mol solution.

Samejima[37] has studied the swelling effect upon silk fibroin by K-salts and obtained following results;

In solution of 0.6 Mol: The order of swelling effect is:

$$NO_3^- > Cl^- > CH_3COO^- > I^- > CNS^- > SO_4^{--}$$

In the solution of 1 Mol: The order of swelling effect is:

$$I^- > NO_3^- > Cl^- > CNS^- > CH_3COO^- > SO_4^{--}$$

In the solution of nitrate, the order of swelling effect by cation is as follows:

in 0.6 Mol solution: $K^+ > Na^+ > NH_4^+$
in 1 Mol solution: $K^+ > NH_4^+ > Na^+$
in 1.3 Mol solution: $NH_4^+ > K^+ > Na^+$

Generally, fish meat protein is dehydrated and coagulated by electrolytes in the high concentration (above 3 Mol). Contrarily, in the low concentration (0.05~0.2 Mol), electrochemical adsorption of ions occurs, while in medium concentration (0.2~1 Mol) both adsorption by electric charge and surface-chemical adsorption occur, respectively, according to concentration of soaking solution.

According to Migita's observation[38] for the setting phenomenon, "Suwari" of fish paste, in medium concentration of electrolytes, ionic hydration by surface chemical adsorption seems to be effective.

The accuracy of the series of swelling effect of various ions in this experiment must be examined in detail in future.

But as far as indicated by the present author's findings, ionic action in the swelling effect of 0.6 Mol solution of various salts seems to be situated between the surface-chemical adsorption and electrochemical adsorption.

From results as above obtained, in the solution of 0.6 Mol, the swelling effects of KCl, KNO_3 and $NaNO_3$ are larger than that of NaCl. Therefore using of those salts seems to be suitable for preventing the formation of "Drip".

The using of KNO_3 or KCl for the soaking solution seems to be rather suitable than the using of NaCl, because of the penetration of those K-salts into red meat (including fish meat) more rapidly[39] and it keeps the bright red colour[40].

2. Conclusion

The relation between the cause of the formation of "Drip" which is separated from frozen fish meat when thawed and Bound Water was studied by vapour tension method using Atka mackerel meat. According to the results obtained, a principal cause of the formation of "Drip" is the dehydrating denaturation of fish meat protein. At this time, a part of water regarded as molecular theoretical Bound Water is set free and the value of $\Delta\overline{F}$ of water in frozen fish meat decreased below that of fresh raw meat.

The method of soaking fish meat in NaCl solution, or in solution of which the value of pH is adequate to prevent the formation of "Drip", was examined. The soaking method is employed for the purpose of the increase of the value of $\varDelta\bar{F}$ of water in fish meat.

From the results obtained, the following conclusions are formulated.

(1) The amount of "Drip" in frozen fish meat increases with falling in freshness of raw material.

(2) The longer the storing time is, the larger the amount of "Drip" becomes.

(3) Water in fish meat from which "Drip" had separated has large value of $\varDelta\bar{F}$, that is to say, the water has strong affinity with fish meat protein.

(4) In the case of soaking of fish meat in NaCl solution, the maximum degree of swelling is shown in the concentration of 3.5% (0.6 Mol).

(5) With regard to the influence of hydrogen ion concentration, in the case of no adding of salts, the minimum value of degree of swelling is shown at pH 4~7, the value of degree of swelling increased at above pH 7 or below pH 4. In the case of adding of salt, the maximum value of degree of swelling is shown in 0.86 Mol NaCl solution having pH 6~7 values.

(6) When the effects of swelling in 0.6 Mol (3.5%) solution of various kinds of salts and chlorides are compared, following series were obtained.

In Na-salts; $CH_3COO^- > NO_3^- > Cl^- > B_4O_7^{--} > SO_4^{--} > PO_4^{---} > C_3H_4(OH)(COO)_3^{---}$

In K-salts; $I^- > Cl^- > NO_3^- > CO_3^{--} > CH_3COO^- > SO_4^{--}$

In chlorides: $K^+ > Na^+ > NH_4^+ > Mg^{++} > Ca^{++}$

The larger the value of the degree of swelling is, the more effective the preventing of the formation of "Drip" is. From this results, the using of K-salts seems to be suitable for preventing the formation of "Drip" than using of Na-salts.

V. VARIATION OF THE AMOUNT OF BOUND WATER DURING SALTING OF FISH MEAT

The author has examined the relation between the total amount of water and the amount of Bound Water in the meat of Atka mackerel (*Pleurogrammus azonus* JORDAN *et* METZ), mackerel (*Scomba japonicus* HOUTTUYN) and squid (*Ommastrephes sloani pacificus* STEENSTRUP) during the dry salting or brine salting by means of the cobaltous chloride method described in the previous Section II.

According to the results obtained, at the time of the decrease of the percentage of the total amount of water in the fish meat by the phenomenon of osmotic dehydration during salting, the amount of Free Water decreased at first, while that of Bound Water increased relatively.

The absolute amount of Bound Water (per gm of dried matter) in Atka mackerel meat decreased with the longer time of salting storage, but those in mackerel and squid meat increased temporarily at the initial period of the salting.

However, the decrease of the absolute amount of Bound Water in Atka mackerel meat is not peculiar to the kind of fish, because the chemical components are different with the kind of fish and there is some variation of the absolute amount of solid matter in the samples of fish meat.

In previous experiment, the penetration of NaCl has not been estimated. In the present experiment, both the estimation of the amount of Bound Water by the cobaltous chloride method, and the quantitative analysis of NaCl which had penetrated into fish meat were made, moreover the variation of the amount of Bound Water in fish meat during salting were reexamined.

In addition, the reabsorption phenomenon of water by "Protein-salts complex", which has been recognized by many investigators[40a)41)] was also examined.

1. Experimental part

(1) *Experiment I (Preliminary experiment)*

(i) Sample

In this experiment, Atka mackerel meat was employed as sample. At sampling, Atka mackerel was cut in both fillets and those fillets were cut a quarter from the lateral line. From back parts of these quarter fillet, some pieces of fish meat (1.5 × 1.5 × 1.5 cm in size) were taken.

(ii) Method of experiment

In this experiment, brine salting with NaCl was employed. The concentration of brine used was 7.2%, 13.7% and 25.4%. Each of 10 pieces of the sample was put into 100 cc. brine each of the various concentrations as above mentioned.

After soaking of those pieces of fish meat, two pieces each were taken out at certain definite intervals. Estimations of the amount of NaCl penetrated into the sample and the amount of water-content were made. The amount of NaCl was estimated by Rusznyak's method[42)].

The amount of water was estimated by drying method as usual (drying temperature was 100°~105°C).

(iii) Results of experiment and discussion

The results obtained are shown in Table 41 and Figs. 32-a, -b, -c.

Fig. 32-a shows the variation of the amount of water-content of Atka mackerel meat during salting, and Fig. 32-b shows that of the amount of NaCl penetrated. In Fig. 32-b the continuous lines represent the amount of NaCl penetrated into the raw sample, and the dotted lines show the amount of NaCl in percentage ratio to dried matter of the same sample.

In Fig. 32-b, the continuous lines represent the amount of NaCl penetrated into the raw sample, and the dotted lines show the amount of NaCl in percentage per dried matter of the same sample.

Fig. 32-c shows the ratio of the amount of NaCl to the sum of the amount of water-content ($m\%$) and NaCl penetrated ($s\%$), ($(s/m+s) \times 100$) in order to pressume the concentration of NaCl in body fluid after Hasegawa[40b)].

As seen in Fig. 32-a, in curve I which shows data of the sample soaked in the most diluted NaCl solution (7.2%) the amount of water-content increased gradually until 3 hours of salting, and thereafter somewhat decreased and then

Table 41. The variations of water-content and NaCl during brine salting of Atka mackerel meat

Time (hrs.)	7.2% NaCl soln. (I)			13.7% NaCl soln. (II)			24.4% NaCl soln. (III)		
	Water-content "m" (%)	NaCl content "s" (%)	$\left(\frac{s}{m+s}\times 100\right)$	m (%)	s (%)	$\left(\frac{s}{m+s}\times 100\right)$	m (%)	s (%)	$\left(\frac{s}{m+s}\times 100\right)$
0	74.78	0.31 (1.21)	0.4	74.78	0.31 (1.21)	0.4	74.78	0.31 (1.21)	0.4
1	75.46	3.88 (15.8)	4.9	73.02	5.37 (19.8)	6.9	66.34	7.85 (23.3)	10.6
3	77.68	4.34 (19.4)	5.3	72.89	8.43 (31.1)	10.4	62.31	11.24 (29.8)	15.3
5	74.40	4.61 (18.0)	5.8	69.57	8.47 (27.8)	10.8	60.35	11.20 (28.2)	15.7
7	76.24	4.70 (19.8)	5.8	70.11	8.26 (27.6)	10.5	59.61	11.12 (27.5)	15.7

Note: The numbers in parentheses show percentage of NaCl per dried matter.

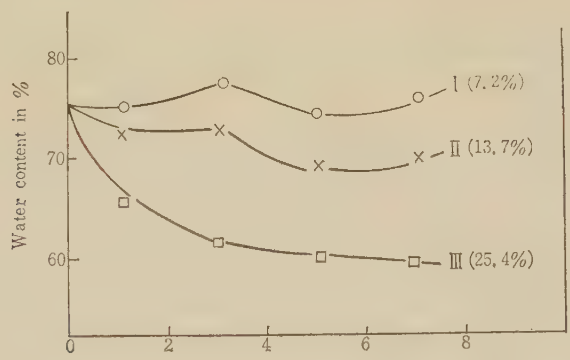


Fig. 32-a. The variation of the amount of water-content (m) during the salting

increased again at 7 hours' salting.

The increase of the amount of water-content in the diluted NaCl solution at initial period of salting is admitted by many investigators[39,41]. This is recently considered to be principally due to lyotropic phenomenon of NaCl ion with water molecule.

In curve II which represents the sample soaked in 13.7% NaCl solution, the amount of water-content decreased gradually until 6 hours, and increased the same as curve I at 7 hours' salting. In curve III which shows the sample soaked in the most concentrated NaCl solution (25.4%), the increase of the amount of

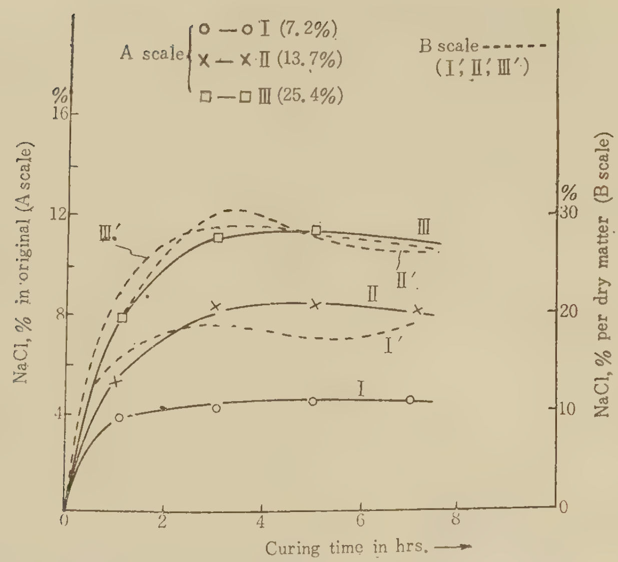


Fig. 32-b. The variation of the amount of NaCl (*s*) during the salting

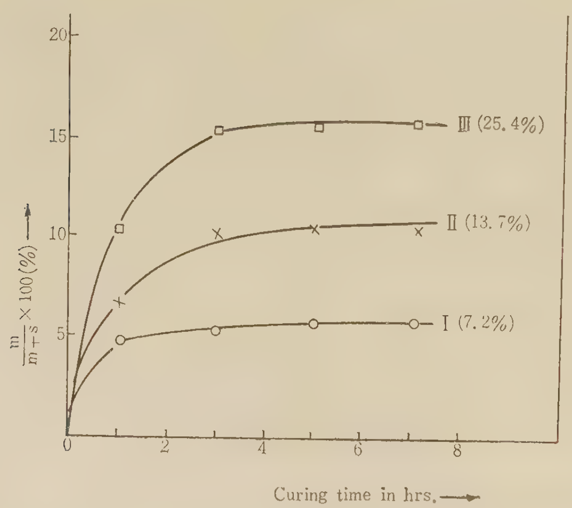


Fig. 32-c. The variation of the value of $(m/m+s) \times 100$ during the salting

water-content was not recognized within the time of the experiment, and it decreased from the initial period of salting. The amount of decreasing of water-content in the sample soaked in 25.4% soln., is much more than that in the samples soaked in 7.2% or 13.7% NaCl solution.

As seen in Fig. 32-b, in curves I, II and III which show the variation of the amount of NaCl in raw fish meat, the amount of NaCl increased rapidly at initial

period of salting until 3 hours, and thereafter that amount in sample which was soaked in 7.2% NaCl solution increased gradually.

The amounts of NaCl in samples which were soaked in 13.7% and 25.4% NaCl solutions reached the maximum point in about 5 hours' salting and thereafter the amount decreased somewhat until 7 hours' salting.

As to the samples soaked in 25.4% NaCl solution, the amount of water-content decreased continuously from the initial period of salting.

Therefore, the decrease of the amount of NaCl as above stated is considered to show the variation of the amount of the dried matter which is caused by the dissolution of NaCl solution soluble protein.

In Fig. 32-b, the variation of the amount of NaCl in relation to the dried matter in fish meat was shown by dotted lines, curves I′, II′ and III′.

In curves I′, II′ and III′, the maximum amount of NaCl is temporarily shown at 3 hours' salting, thereafter the amount decreases and then increases. Those curves I′ and II′ have a tendency to correspond with curves I and II which show the variation of the amount of water-content in raw meat respectively. As to the concentration of NaCl in the body fluid, which is shown in Fig. 32-c, an apparent constant was shown in 3~4 hours; after 7 hours' salting the amount of NaCl in the body fluid of fish meat soaked in 7.2% NaCl solution was about 5.3%, in 13.7% NaCl solution, about 10.6% and in 25.4% NaCl, about 15.7%.

As above stated, after the amount of NaCl penetrated reached apparent constant in 3 hours' salting, the variation of the amount of water-content and that of NaCl penetrated in proportion to the dried matter of fish meat have the same tendency.

From the results as above obtained there is clearly a correlation between the penetrated amount of NaCl into, and the amount of the withdrawal of water from the fish meat.

That is to say, after the amount of NaCl penetrated attains apparent constant, withdrawal of body fluid to the outside of the fish meat occurs, at the same time NaCl diffuses with water to outside brine; contrarily, when water is absorbed to the inside of the fish meat, at the same time NaCl penetrates into the fish meat, so the definite ratio of NaCl to water is always kept.

However, it is noteworthy that the maximum amount of NaCl in body fluid is always less than the amount of NaCl in outside brine. As to those facts, Reay[41)] and Hasegawa[40b)] have obtained the same results respectively.

Hasegawa has studied the penetration of NaCl using about 4.3 gm of tuna meat. According to his observations, the amount of NaCl penetrated into the meat reached apparent constant after 20 hours' salting and did not reach the same degree of concentration of NaCl as the used brine even after 100 hours' salting.

The fact that the concentration of NaCl in the outside solution (brine) is not the same as that of NaCl in the body fluid of fish meat nor even nearly resembling it, leads to the following consideration: The total amount of water in the body fluid was not act as solvent for NaCl after the amount of NaCl penetrated attains apparent constant, and a part of the water only dissolves

Table 42. Water distribution in salted Atka mackerel meat after the penetration of NaCl attained to apparent constant

Time (hrs.)	7.2% NaCl soln. (I)				13.7% NaCl Soln. (II)				25.4% NaCl soln. (III)			
	Total Water	Free Water	Bound Water		T.W.	F.W.	B.W.		T.W.	F.W.	B.W.	
	%	%	%	"g"	%	%	%	"g"	%	%	%	"g"
3	77.7	55.8	21.9	0.98	72.9	53.0	19.9	0.74	62.3	33.0	29.3	0.78
5	74.4	59.4	15.0	0.59	69.6	53.3	16.3	0.54	60.4	32.9	27.5	0.69
7	76.2	60.6	15.6	0.69	70.1	52.3	17.8	0.59	59.6	32.6	27.0	0.67

Remarks: The concentration of NaCl in the fluid of fish meat was supposed to $(S/\text{F.W.}+S)\times 100=7.2\%$ or 13.7%, or 25.4% respectively.

NaCl penetrated and keeps the definite concentration in the body fluid of fish meat.

Table 42 shows the values of the theoretical amounts of Free Water and Bound Water which were calculated from the results obtained in Table 41, supposing that the penetrating amount of NaCl reaches apparent constant after 3 hours' salting and the concentration of NaCl in the body fluid is the same as that of NaCl in the outside brine.

In Table 42, the value of "*g*" represent the theoretical amount of Bound Water per gm of dry matter of the samples. As the concentration of the outside brine is diluted by water withdrawn from the fish meat, the practical values of the amounts of Free Water and Bound Water may differ somewhat from the values given in Table 42.

As seen in Table 42, the calculated amounts of Bound Water in fish meat soaked in A solution (7.2% NaCl solution) and B solution (13.7% NaCl) are about 15~22% in raw material, and it is about 17~30% in the fish meat soaked in C solution (25.4% NaCl). Those calculated amounts of Bound Water are 0.5~1 gm per gm of the dried matter. Those values agree with the maximum amounts of Bound Water which were estimated by the cobaltous chloride method for salted squid and mackerel meat as reported in the previous Section II.

It is impossible to discuss the variation of the amounts of Bound Water from the results as above obtained since they contain a few presumptions. However, it is clear that there is some amount of Bound Water which does not dissolve NaCl penetrated into the fish meat.

As seen in curves II′ and III′ of Fig. 32-b, the proportion of the increasing of NaCl relative to the dried matter of fish meat soaked in 25.4% NaCl solution was larger than that of the fish meat soaked in 13.7% NaCl solution at the initial period of salting. There was almost no difference between the amount of NaCl penetrated into the fish meat which was soaked in those two solutions after 3 hours' salting. Therefore, in this experiment, if the larger amount of NaCl penetrated into the fish meat which was soaked in higher concentration of NaCl solution, the larger amount of water is withdrawn correspondingly from the fish meat.

On the contrary, if the smaller amount of NaCl penetrated into the fish

meat which was soaked in lower concentration of NaCl solution, a smaller amount of water is withdrawn correspondingly from the fish meat. There seems always to be a definite ratio of the penetrating amount of NaCl to the amount of water withdrawn to the outside.

According to Reay[41)], Callow[43)] and Yamada[44)] who have studied on the phenomenon of reabsorption of water by "Protein-salts complex", if an excessive amount of NaCl penetrates into the fish meat which was soaked in high concentration of NaCl solution, the fish meat protein peptizes and again absorbs water from outside.

In this experiment, the increasing of the amount of water-content in the fish meat soaked in 7.2% NaCl solution or 13.7% NaCl solution was recognized after 5~7 hours' salting. This agrees with the phenomenon of reabsorption of water by "Protein-salts complex". In this case, the amount of NaCl penetrated into the fish meat increases correspondingly with the reabsorption of water. The detail of this phenomenon will be discussed in the following experiment.

(2) *Experiment II*

(i) Sample; 12 Atka mackerel were viscerated and cut to fillet, and one side of the fillet was skinned.

(ii) Method of experiment

For brine salting of samples, brine of NaCl Bé 5° and Bé 11° were employed. For dry salting, solid NaCl was added to 25% of the weight of the sample. Each 7 pieces of fish fillet were employed for both salting methods. After salting each one piece was taken after 1, 2, 4, 5, 7, 9, and 11 days. About 2 gm of meat were taken from back part of the fish fillet for the estimations of the amount of water, NaCl and Bound Water.

(iii) Results of experiment and Discussion

The results obtained are shown in Table 43 and Fig. 33-a~33-d.

In every Fig., curves I, II and III show the results in brine salting Bé 5°, Bé 11° and dry salting respectively. As seen in curve I of Fig. 33-a, showing the variation of the amount of water-content during salting, the water-content in the sample soaked in brine of Bé 5° decreased slightly until the 4th day of the initial period of salting; it increased remarkably in 4~6 days period and swelled, then it decreased again after 6 days.

As seen in curve II of Fig. 33-a, the water-content in the sample soaked in brine of Bé 11° decreased rapidly to 55% in one day and showed constant values for 5 days; then it increased again after 5 days salting, and became about 77.7% after 11 days.

As seen in curve III of Fig. 33-a, the water-content in the sample by dry salting showed a tendency almost the same as curve II (brine salting, Bé 11°), but the decreasing of the water content is smaller than that of the sample soaked in the brine at Bé 11°.

Fig. 33-b shows the variation of the amount of NaCl in the original samples during the salting.

As seen in curve I of this Fig., the amount of NaCl in the sample soaked

Table 43. The variations of the amounts of water and NaCl during salting of Atka mackerel meat

Conc. of NaCl	Items		1 day	2 days	4 days	5 days	7 days	9 days	11 days	Raw meat
Bé 5°	Total Water content	(%)	76.00	75.75	74.51	88.30	86.22	77.36	78.24	81.57
	Free Water content	(%)	61.60	68.22	69.16	85.80	85.40	71.70	68.66	71.56
	Bound Water content	(%)	14.40	7.53	5.35	2.50	0.82	5.66	9.58	10.01
	Bound Water content	(*g*)	0.60	0.31	0.21	0.21	0.06	0.25	0.44	0.55
	NaCl (%)	(*S*)	1.95	1.59	2.24	1.46	2.09	2.27	2.28	0.22
	NaCl per dry matter	(%)	8.12	6.55	8.79	12.5	15.2	10.0	10.5	1.19
	$\left(\frac{S}{F.W.+S}\times 100\right)$	%	3.17	2.33	3.24	1.7	2.45	3.17	3.32	0.31
Bé 11°	T.W.	(%)	54.95	54.29	56.56	52.22	69.68	75.72	77.68	
	F.W.	(%)	39.15	20.49	18.36	6.32	63.01	66.47	73.88	
	B.W.	(%)	15.80	33.80	38.20	45.90	6.67	9.25	3.80	
	B.W.	(*g*)	0.35	0.74	0.88	0.96	0.22	0.38	0.17	
	NaCl	(*S*)	2.13	3.03	5.00	3.28	5.36	5.31	5.05	
	NaCl per d.m.	(%)	4.73	6.44	11.5	6.86	17.7	21.8	22.6	
	$\left(\frac{S}{F.W.+S}\times 100\right)$	%	5.45	14.8	27.2	52 ?	8.5	8.0	6.84	
Dry salting	T.W.	(%)	74.35	69.84	60.51	61.16	66.03	65.27	76.48	
	F.W.	(%)	58.95	57.14	38.61	47.56	63.31	53.17	67.65	
	B.W.	(%)	15.40	12.50	21.90	13.60	2.72	12.1	6.80	
	B.W.	(*g*)	0.60	0.42	0.74	0.35	0.08	0.35	0.29	
	NaCl	(*S*)	2.07	6.41	8.34	9.27	8.21	12.16	11.32	
	NaCl per d.m.	(%)	8.07	21.2	21.2	23.9	24.2	35.0	48.2	
	$\left(\frac{S}{F.W.+S}\times 100\right)$	%	3.52	11.2	14.6	19.5	13.0	22.9	16.3	

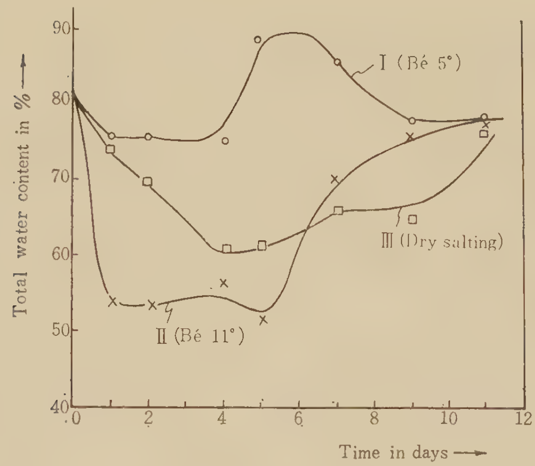


Fig. 33-a. The variation of the amount of water-content (m) during the salting

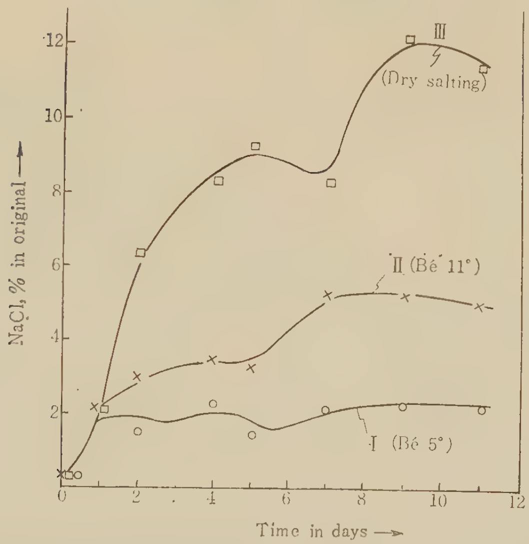


Fig. 33-b. The variation of the amount of NaCl (S) in the original samples during the salting

in brine of Bé 5° showed about 2% at first day of salting, and thereafter showed almost constant value.

As seen in curve II of Fig. 33-b, the amount of NaCl in the sample soaked brine of Bé 11° showed constant value of 3~3.5% at the 2nd~5th day of the salting, and increased to about 5.4% at the 7th day, then decreased somewhat until the 11th day.

As seen in curve III of Fig. 33-b, the amount of NaCl penetrated into the sample by dry salting was larger than that of samples which were soaked in

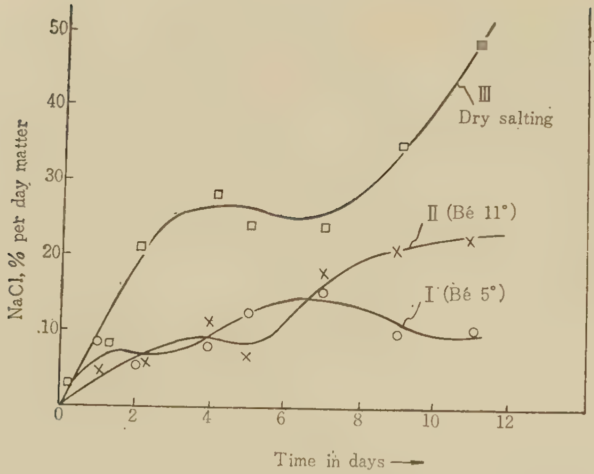


Fig. 33-c. The variation of the amount of NaCl (S) in the dried matter during the salting

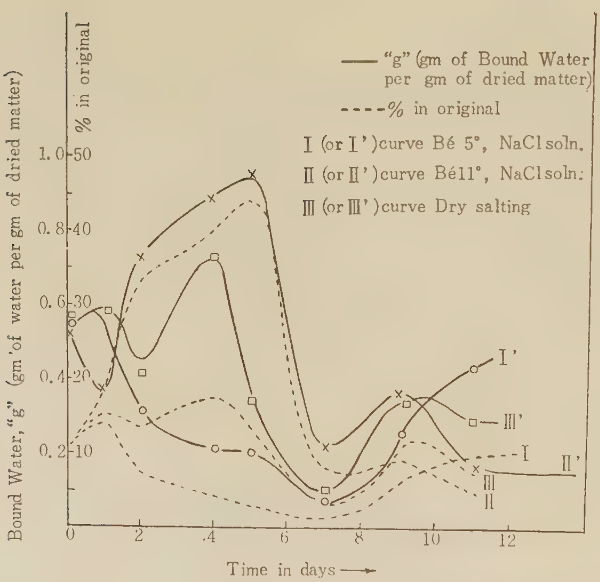


Fig. 33-d. The variation of the amount of Bound water estimated by cobaltous chloride method during the salting of Atka mackerel meat

brine of Bé 5° or Bé 11°. The tendency of the increase resembled that of the samples by brine salting. In the 4~6 days period the constant value of the first stage, about 8.2%, was shown, and at the 9th day it increased rapidly to above 12%; thereafter the amount of NaCl decreased.

Comparing Fig. 33-b with Fig. 33-a, in the case of salting of brine of Bé 11° and dry salt the amount of NaCl increased rapidly at the time of initial decreasing of the water-content, and the amount of NaCl showed first stage of constant value at the time when the water-content began to increase.

It increased at the time of increasing of the water-content and attainend to the second stage of constant value of the amount of NaCl.

Fig. 33-c shows the variation of the amount of NaCl in the dried matter of fish meat during salting.

The tendency of the variations of NaCl which were shown in curves I, II and III is almost the same as that of Fig. 33-b. But curve I of Fig. 33-b which showed the variation of the amount of NaCl in the original sample soaked in brine of Bé 5° did not show any more remarkable variation than curve I of Fig. 33-c; and it showed a constant variation.

On the other hand, after 4 days period of salting, the tendency of curve I of Fig. 33-c showing the variation of the amount of NaCl in dried matter of sample was shown as parallel in comparison with that of curve I of Fig. 33-a which showed the variation of the water-content in original sample. Such a relation between curve I of Fig. 33-c and curve I of Fig. 33-a was also shown in curve II of each Fig. showing the samples soaked in brine of Bé II° and in curve III of each Fig. of the dry salted samples.

Interpreting the results as above stated, if the reabsorption of water is due to the absorption by "Protein-salts complex" formed in the fish meat, NaCl penetrates into the meat, at the same time the infiltration of water into the fish meat from the outside solution occurs.

Therefore the larger the amount of absorbed water becomes, the larger the amount of NaCl penetrates into the fish meat.

In this case, therefore, it is impossible to consider that water only infiltrates into the fish meat, contrarily that NaCl only diffuses out.

At the initial period of salting when the dehydration from the fish meat is severe, the penetration of NaCl is clearly owing to osmotic dehydration and osmotic invasion of NaCl; finally the amount of penetrated NaCl reaches a constant. But in fish meat soaked in the brine of Bé 5°, it is considered that the increase of water-content is owing to the lyotropic phenomenon by electrolyte ion. This will be discussed later on.

Fig. 33-d shows the variation of the amount of Bound Water estimated by cobaltous chloride method during the salting of fish meat by various methods.

In the Fig. 33-d the dotted line shows the percentage of Bound water in the original sample, and the continuous line shows the absolute amount of Bound Water (g) per gm of the dried matter of the sample. The variation of the amount of Bound Water in the original shows the relative reverse tendency in respect to that of the total amount of water which is shown in Fig. 33-a.

As seen in curve I of Fig. 33-d which shows the fish meat soaked in the brine of Bé 5°, the amount of Bound Water increased, and the total amount of water decreased correspondingly on the first day of salting.

With 1~4 days when the total amount of water showed almost constant,

the amount of Bound Water somewhat decreased. Within 4~7 days which is in the period of increasing of the total amount of water, the amount of Bound Water decreased farther. After 7 days which is in the period of decreasing of the total amount of water, the amount of Bound Water increased again. Similar processes are indicated in curves II and III also. Therefore, each curve of Fig. 33-d which shows the variation of the amount of Bound Water in original sample corresponds to each curve of Fig. 33-a showing the variation of the total amount of water in original sample.

It seems as a peculiar property of the variation of the amount of Bound Water, that in curves II and III which show data for the samples soaked in brine of Bé 11° and dry salted respectively, the amount of Bound Water decreased at the increasing period of the total amount of water after 5 days' salting, but it increased somewhat at the 8~9th day and then decreased again at 10th day.

In curve I of Fig. 33-d, on the other hand, at the decreasing period of the total amount of water after 7 days' salting, the amount of Bound Water increased relatively.

The continuous line in Fig. 33-d which shows the variation of the absolute amount of Bound Water per gm of the dried matter resembles almost the dotted line of the variation of the amount of Bound Water in original samples.

In the previous section II the author has observed that the increase of the absolute amounts of Bound Water "*g*" in the salted squid and mackerel was the same as in the variation of Bound Water "*g*" as above stated, but in that time, the increase of that amount in salted Atka mackerel meat has not been clearly observed.

In the present experiment, the increase of the amount of Bound Water in Atka mackerel meat was clearly observed at the initial period of salting.

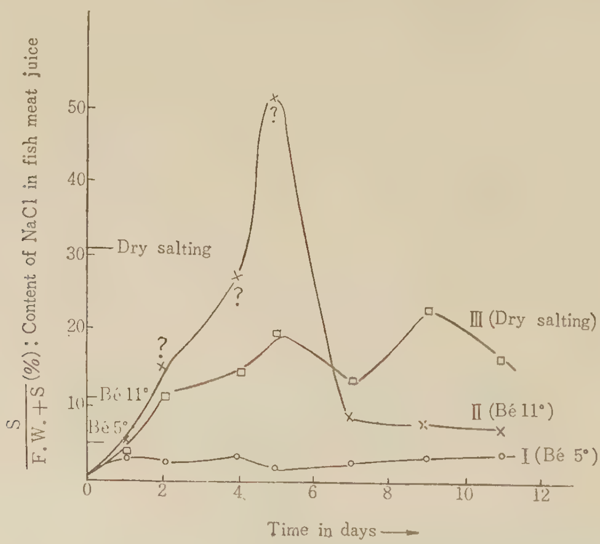


Fig. 34. The variation of the value of $(S/F.W.+S)\times100$ during the salting of Atka mackerel meat

From the assumption that NaCl which penetrated into fish meat is not dissolved in Bound Water, but dissolves in Free Water, the author has calculated the concentration of NaCl in the body fluid of fish meat from the values written in Table 43; the results are shown in the same Table and Fig. 34.

As seen in curves I and III of Fig. 34, which represent the samples soaked in the brine of Bé 5° and dry salted respectively, the concentration of NaCl in the body fluid increased with the longer time of salting.

Moreover, that concentration of NaCl in fish meat soaked in the brine of Bé 5° showed 3.3% in maximum; the concentration in the dry-salted fish meat showed 23% in maximum.

The concentration of NaCl of the outside brine of Bé 5° is equal to about 5%. In the case of dry salting, if all the NaCl used penetrates into the fish meat the concentration of NaCl in body fluid became above 30%. The value of concentration of NaCl as above obtained is less than that of the concentration of outside solution.

As seen in curve II of Fig. 34 which is for the fish meat soaked in brine of Bé 11°, the concentration of NaCl in the meat increased remarkably to 14~52% at 2nd~5th day of salting. Supposing the concentration of NaCl in the outside solution is about 11%, the concentration of NaCl stated above seems to be abnormally high. This fact shows that the amount of Bound Water at 2nd~5th day estimated by cobaltous chloride method is too much.

The fact that the absolute amount of Bound Water per gm of the dried matter of the sample has increased in the initial period of the salting was often recognized in the previous experiments. From this fact, the abnormal phenomenon of the increase as above stated seems to be owing to the estimating conditions of Bound Water by the cobaltous chloride method.

For example, when salts are contained in the sample (containing cobaltous chloride as a kind of salt[45)]), the value of apparent amount of Bound Water is considered to increase owing to combining ability of salt ion for the fish meat protein and the ability of the ion itself to water holding.

As seen in those experiments, on the other hand, the fact that a part of fish meat protein dissolves out into the outside solution in the initial period of brine salting and therefore the decrease of the solid matter occurs is considered to be one cause of the abnormal phenomenon of the increase of the absolute amount of Bound Water.

Therefore it may be somewhat unreasonable that the variations of the amount of Bound Water shown in each curve of Fig. 32-d should be regarded as the true variation of the amount of Bound Water.

However, when the estimated values of the amount of Bound Water for the sample soaked in the brine of Bé 11° for 2, 4 and 5 days are excepted, it is generally better to consider that the absolute amount of Bound Water "*g*" decreases until 7 days of salting.

In the sample soaked in the brine of Bé 11° and the sample which was dry salted, until 5 days, water withdrawn out into the outside solution. At the same time NaCl penetrated into the fish meat by osmotic action corresponding to the concentration of NaCl in outside solution, and reached to apparent

constant. During the time until that when the amount of NaCl reached the apparent constant the amount of Bound Water estimated by cobaltous chloride method increased apparently owing to the increase of the amount of NaCl penetrated and owing to the efflux of NaCl solution soluble matter from fish meat protein into the outside solution.

As the result of the accumulation of NaCl penetrated into the fish meat, it takes part in the effect of "salting out" and seems to precipitate the fish meat protein.

After the time when the amount of Bound Water reached the apparent constant, the fish meat protein peptizes and again absorbs the outside solution. At that time NaCl and water in the outside solution seem to penetrate into the fish meat in keeping with a certain definite concentration.

When the reabsorption of water occurs at the peptization of the fish meat protein, the amount of Bound Water as estimated by cobaltous chloride method seems to increase again resultant from the increase of the hydrating affinity of the peptized fish meat protein with water molecule. At the 11th day when the reabsorption phenomenon attained a constant, the apparent amount of Bound Water decreased owing to the increasing of the dehydrating power caused by NaCl penetrated.

In the case of fish meat soaked in the brine of Bé 5°, as previously stated, within the 4th day of the salting, the amount of water in the sample decreased; after the 4th day the amount increased rapidly, the fish meat came to contain a larger amount of water than that before the salting, and the meat was recognized to swell remarkably. The brine of Bé 5° contains about 5% of NaCl in the solution. Generally in such a diluted solution containing 5% NaCl (0.8 Mol), surface chemical hydration by electrolyte ion plays the leading rôle[39]. After the 4th day of the salting, the abnormal increasing of the amount of water may be due to the increase of the hydration by salt ion adsorbed on the surface of the fish meat protein peptized.

2. Conclusion

During the initial period of the salting of fish meat, the osmotic dehydration from the fish meat and penetration of NaCl in it are recognized; at the same time the apparent amount of Bound Water as estimated by cobaltous chloride method decreased. This finding seems to be due to the increase of the hydrating affinity, accompanied by the increase of salt-content in the sample or it may be due to the estimating method itself. In practice, during the time when the osmotic phenomenon is attaining a constant, the amount of Bound Water decreases owing to the increasing of the dehydrating power caused by NaCl penetrated.

During that time, the fish meat protein seems to denaturate by the effect of "salting out", and with the longer time of salting, the peptization of fish meat protein occurs. Then the apparent amount of Bound Water was recognized to increase owing to the absorption of water from the outside solution.

In case of the dilute brine, the abnormal absorption of water occurs owing to the effects of lyotropic action and surface chemical adsorption of salt ion.

VI. RELATION BETWEEN THE DECOMPOSITION OF FISH MEAT PROTEIN AND THE AMOUNT OF BOUND WATER DURING THE AUTOLYSIS OF FISH MEAT

The author has observed that the amount of Bound Water estimated by cobaltous chloride method or electrical resistance method in fish meat decreased during autolysis of putrefaction in the experiments described in Section II.

In the present experiment, the autolytic actions of raw fresh Atka mackerel meat and frozen same meat after thawing were observed. From the results obtained, the behaviour of Bound Water in the samples is discussed.

1. Experimental part

1. *The relation between the autolysis of raw fresh fish meat and of frozen fish meat and the amount of Bound Water*

Previously Amano[46] has investigated the rigor motis of frozen whale meat after thawing, and observed that the glycolytic action which was stopped during the freezing of whale meat clearly recommenced at the thawing.

It is well known that the autolytic action in fish meat is very slow when the fish meat is stored at comparatively low temperatures. Particularly, even if there is some amount of Free Water in frozen material, the enzymic action is stopped entirely.

It was clearly observed that some amount of "Drip" separated owing mainly to the denaturation of fish meat protein after the thawing of frozen meat as stated in the previous Section IV. There are various types of glycolytic action during autolysis. According to Yamada[47], glycogen which was considered as being combined with tissue protein phosphorates at first in that glycolysis, and at this time the amount of Bound Water varies naturally.

As seen clearly in the present author's experimental results which were previously obtained, the amount of Bound Water of frozen fish meat became less than that of the raw fresh fish meat in comparison at the same water-activity "a". Therefore the progress of autolytic action of frozen fish meat after thawing was considered to be not the same as that of raw fresh fish meat.

2. *Method of experiment*

In this experiment, three Atka mackerel were employed as the samples, one was employed as it was and the other two were quickly frozen at $-30°C$ and stored during one week and two weeks at $-18°C$ respectively. After storage, the meat was taken and homogenized with a blendor and antiseptized with toluene, and left alone at $32 \pm 1°C$.

After autolysis of the fish meat, the amounts of amino acid nitrogen (after Pope-Steven's method), volatile basic nitrogen (after Weber and Wilson), 1% NaCl soluble protein nitrogen and the value of pH of the sample were estimated at certain definite intervals. The results obtained are described below.

The chemical components of raw fresh meat of Atka mackerel employed as sample were as follows: water-content 64.86%, ash 1.04%, crude fat 16.6%, total nitrogen 2.8%, and crude protein 17.5%.

3. *Results of experiment and discussion*

The results obtained are shown in Tables 44~46 and Figs. 35~38.

Fig. 35 shows the variation of the values of pH during the autolysis of the samples. Fig. 36 shows the variation of the amount of 1% NaCl soluble protein nitrogen. Fig. 37 shows the variation of the amount of volatile basic nitrogen and amino acid nitrogen. In each of the Figs., curve I shows data on the sample of raw fresh Atka mackerel, curve II shows the sample which was frozen at −30°C and stored at −18°C for one week, curve III shows the sample frozen at −30°C and stored at −18°C for two weeks.

As seen in Fig. 35, in curve I of raw fresh meat, 6.8 of pH value at initial period of autolysis, decreased gradually with the lapse of time, and showed 6.15 of minimum value after 51 hours, and then increased again with the longer time of duration. From the results as above stated, it was considered that the normal glycolysis was progressing as well as raw fresh meat. In curves II or III which showed the fish meat frozen then stored for one week or two weeks respectively, no remarkable variation of the values of pH was observed as there

Table 44. Autolysis of raw Atka mackerel meat

Time (hrs.)	pH	1% NaCl -soluble-N (%)	V.B.-N (mg%)	Amino-N (mg%)	V.B.-N+Am.-N (mg%)
0	6.80	0.43	9.4	21.0	30.4
3	6.82	0.37	8.9	25.2	34.1
21	6.80	0.16	16.3	25.2	41.5
27	6.70	0.13	16.8	27.4	44.2
45	6.18	0.13	19.5	23.6	43.1
51	6.15	0.10	22.3	23.0	45.3
74	6.50	0.11	27.2	30.8	58.0
93	7.15	0.13	41.6	39.2	80.8
117	7.20	0.12	45.0	58.8	103.8
123	7.25	0.12	47.5	60.2	107.7

Table 45. Autolysis of Atka mackerel meat frozen at −30°C and stored at −18°C for one week

Time (hrs.)	pH	1% NaCl -soluble-N (%)	V.B.-N (mg%)	Amino-N (mg%)	V.B.-N+Am.-N (mg%)
0	6.86	0.34	8.9	16.0	24.9
26	7.00	0.16	9.4	24.1	33.5
27	7.10	0.12	14.2	21.0	35.2
45	6.90	0.14	23.9	23.0	46.9
51	6.80	0.15	24.9	25.2	50.1
69	6.80	0.13	25.4	24.1	49.5
75	6.70	0.10	26.4	25.6	52.0
77	6.83	0.12	35.3	26.6	62.9
93	7.10	0.13	38.3	30.8	69.1

Table 46. Autolysis of Atka mackerel meat frozen at −30°C and stored at −18°C for two weeks

Time (hrs.)	pH	1% NaCl -soluble-N (%)	V.B.-N (mg%)	Amino-N (mg%)	V.B.-N+Am.-N (mg%)
0	6.50	0.36	13.4	28.2	41.6
18	6.60	0.18	15.2	30.7	45.9
24	6.70	0.13	18.5	30.8	49.3
42	6.40	0.15	20.8	35.9	56.7
48	6.70	0.11	22.4	33.4	55.8
72	6.80	0.12	26.4	34.6	61.0
112	6.34	0.12	31.5	39.2	70.7

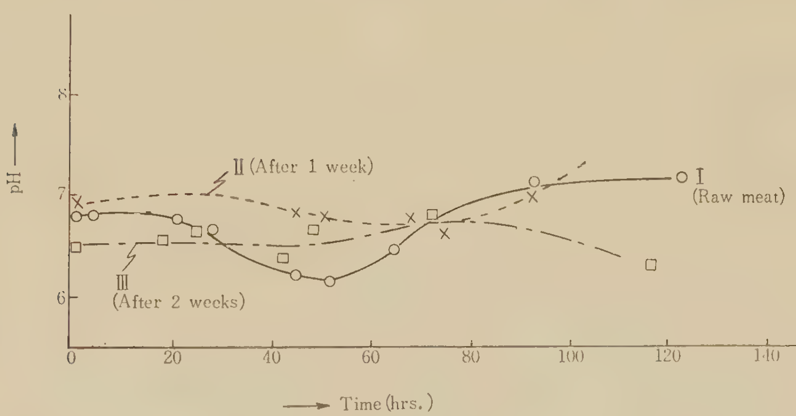


Fig. 35. Variation of pH values during autolysis of Atka mackerel meat

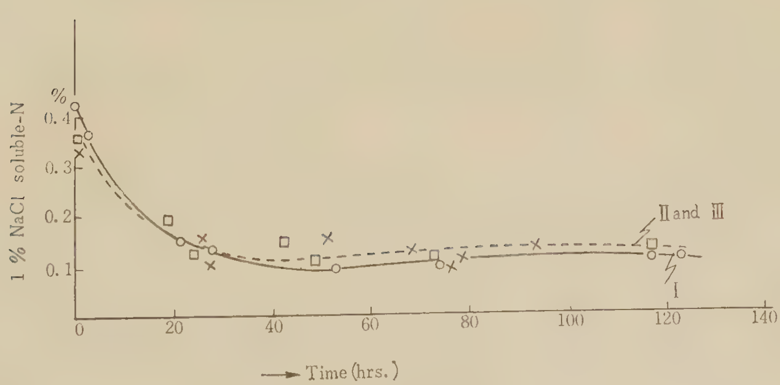


Fig. 36. Variation of the amount of 1% NaCl soluble protein nitrogen during autolysis of Atka mackerel meat

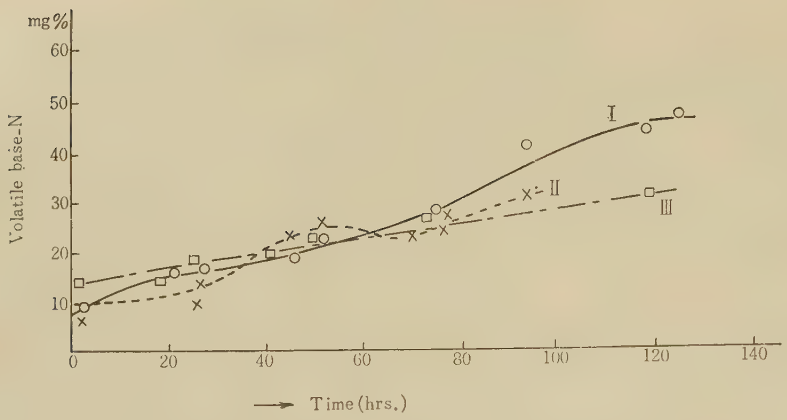


Fig. 37-a. Variation of the amount of volatile basic nitrogen

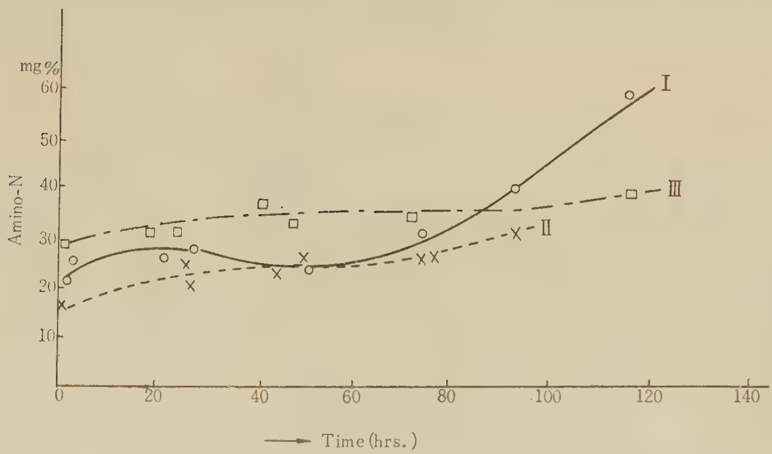


Fig. 37-b. Variation of the amount of amino acid nitrogen

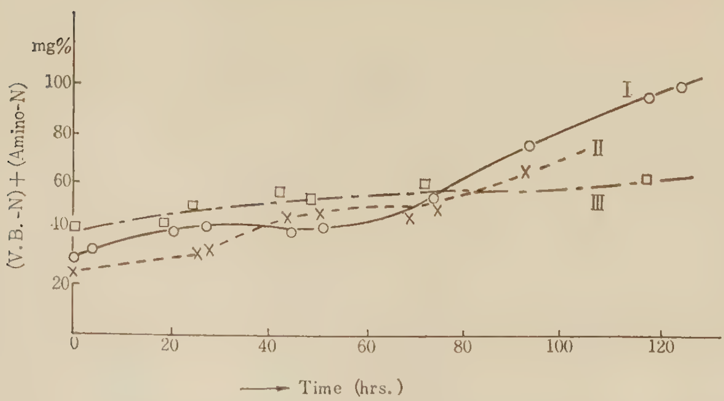


Fig. 38. Variation of the sum of volatile basic and amino acid nitrogens

was in fresh meat. Particularly, in curve III, the initial decrease of the pH value was not observed. Therefore the glycolytic action in th frozen fish meat was not recognized to progress as in the case of the raw fresh meat.

As seen in Fig. 36 showing the variation of the amount of 1% NaCl soluble protein nitrogen in frozen fish meat, the amounts showed less value than those of the raw fresh meat. This is perhaps due to the denaturation of fish meat protein during the freezing storage. After thawing, the amount of 1% NaCl soluble protein nitrogen decreased comparatively until 20 hours' autolysis, and thereafter no remarkable variation was observed with the longer duration of autolysis. Generally, the increase of the amount of 1% NaCl soluble protein nitrogen owing to decomposition of fish meat protein in putrefaction was recognized. But in autolysis, the decrease of the amount of 1% NaCl soluble protein nitrogen as above observed is perhaps due to the denaturation of fish meat protein in the various glycolytic actions such as the formation of lactic acid, the increase and decrease of A.T.P. level and the decrease of SH radical as reported by Yamada[48)] and Amano[46)].

As seen in Fig. 37-a and Fig. 37-b showing the variations of the amounts of volatile basic nitrogen and amino acid nitrogen respectively, in curve I of raw fresh fish meat, the amounts of both nitrogens increased very gradually until about 50 hours' autolysis, and thereafter increased comparatively rapidly. At that time of 50 hours' autolysis, the value of pH of raw fish meat showed the minimum value as above stated.

From the results obtained, it is considered that there are two different stages where the fish meat is decomposed separately, (1) in the decreasing period of the value of pH and (2) in the increasing period. In curves II and III which showed for the samples which were stored for one week or two weeks respectively after freezing, the increase of the amounts of both volatile basic nitrogen and amino acid nitrogen of both samples is slower than that of the raw fish meat. But the tendency of curve II seemed to be almost the same as that of curve I.

As seen in Fig. 38 which showed the variation of the sum of the amounts of volatile basic nitrogen and amino acid nitrogen, the value for frozen fish meat was less than that for the raw fish meat.

Here, autolytic velocity constant (K) was calculated by applying mono-molecular autocatalytic reaction equation (9) after Kimata[49)]:

$$\log y/A-y=Kt+c \qquad (9)$$

where A is the maximum amount of the sum of volatile basic nitrogen and amino acid nitrogen produced, y is the amount of increase at every time "t" and c is a constant.

The value of $K\times10^4$ was 202 for the samples of both the raw fish meat and the frozen fish meat stored one week. The value of $K\times10^4$ was 157 for the sample of frozen fish meat stored two weeks. From the values obtained, it was recognized that the longer the storing time became, the less the autolytic velocity constant was.

From the results just described the decomposing velocity of fish meat protein

by autolysis for the frozen sample is clearly slower than that for the raw sample. The reason for the difference as stated is due to the denaturation of the substrate (the denaturation of fish meat protein) or to the decreasing of enzymic action by the freezing. But, generally, it is recognized that the enzymic action which has been restrained by lower temperature is brought back to normal action when the surrounding temperature become favorable. Therefore the difference of the values of autolytic velocity constant as above stated is perhaps mainly due to the denaturation of fish meat protein.

This is conducted from the previous experimental results IV, that water in the frozen fish meat has less differential molal free energy than that in the raw fish meat, therefore the amount of Bound Water in the frozen fish meat (molecular theoretical Bound Water having the same water-activity as water in the raw fish meat) is less than that in the raw fish meat. That is to say, the amount of Free Water in the frozen fish meat is much greater than that in the raw fish meat, and the frozen fish seems to have lyophobic properties (This was clearly recognized in Tarr's results[31]).

Considering from the study of Sponsler, Bath and Ellis[20], in the raw fish meat within the amount of the water-content regarded as molecular theoretical Bound Water "*g*", three molecules of water seem to be combined with amino radical, four molecules of water with carboxyl radical, three molecules with hydroxyl radical and two molecules with imino radical.

On the other hand, in the frozen fish meat, within the same amount of the water-content "*g*", much water whose molecules seem to be distributed as above stated, accumulated on the surface of radicals as stated.

As the result, the layer of hydration on the surface of protein molecule increases, and the number of polar radicals decreased, therefore, the frozen fish meat is less decomposable by enzyme than raw fish meat.

In consequence of various glycolytic actions in the autolysis of raw fish meat and the frozen fish which was stored for one week, in the variations of the values of pH or the amounts of volatile basic nitrogen and amino acid nitrogen, there seem to be two stages being separated that within, and that after the period 50 hours' autolysis. In the first stage the enzymic action is slow.

As Yamada[47] stated, if the increase or decrease of lactic acid and the increase of inorganic phosphorus in the glycolytic action are considered the apparent amount of Bound Water seemed to increase in the first stage of autolysis and to decrease in the second stage with the longer time of the decomposition of fish meat protein.

2. Conclusion

From results obtained, it is clear that various phenomena in autolytic action which are observed in raw fish meat are restrained without disappearance during the freezing storage of fish meat.

The duration and the strength of the autolytic action is clearly different with the kind of fish, therefore various factors concerning the autolysis will be kept or not during the freezing storage differing from the kinds of fish. With the denaturation of fish meat protein by the longer time of freezing storage, the

various phenomena in autolytic action will vary when the frozen fish meat is thawed, and the apparent amount of Bound Water seemed to increase in the first stage of autolysis and to decrease in the second stage with the longer time of the decomposition of fish meat protein.

VII. GENERAL DISCUSSION

As to Bound Water, there are nowadays three definitions[1a]: "biological Bound Water" which is studied from the standpoint of biology and physiology, "colloidal Bound Water" from the standpoint of colloidal chemistry and "molecular theoretical Bound Water" in the molecular theory. Generally speaking, "biological Bound Water" means the minimum amount of water-content in living matters necessary to maintain their lives; "colloidal Bound Water" may be interpreted as water which necessary to maintain the stable colloidal systems, "molecular theoretical Bound Water" may be taken to mean the amount of water-content to maintain the the stable construction in protein molecules which is a main component of fish muscle.

Recently, Kuprianoff[50] has discussed on "Bound Water in Foods", in which he suggested that generally there is no "free water" in foods, the water present is always "bound" in some way to one or other of its components. According to his opinion, only "surface water" on foods which comes from outside by water condensation or washing, *etc.*, could be considered as "true free water" so long as it has not mixed or reacted in any way with the surface components.

The water in foods, except "surface water" stated above, is a complex matter and depends on many different factors, and the complex water solution present in solid foods may not be bound to colloid particle by hydration, but also adsorbed on the intercellular surfaces and in internal capillaries; and different solutions with different concentrations could be present at different points as long as the structure of the product is not damaged or disrupted.

As far as the water in foods concerned, it may be true; but the next difficulty in the definition of Bound Water arises because it is based on the method used for determination. Which to use among those conceptions on Bound Water as stated above, should be chosen from the samples to be used (including living things), the purposes of the studies and method of estimating the amount of Bound Water in each special field.

In the present paper, following on the historical advancement of Bound Water research, the author has studied the behaviour and the properties of Bound Water in the muscle of fish, mollusc and crustacean (after death) on the basis of the definitions of "colloidal Bound Water" and "molecular theoretical Bound Water" as above described.

In these studies, the author has employed the cobaltous chloride method offered by Hatschek[14a] (Hatschek's method) and later modified by Oyagi[14b] (Oyagi's method), in making estimates of Bound Water in fish muscle.

By the use of those methods, estimate are made of the maximum value of the amount of Bound Water at the point B.P. where the color of a piece of dyed

sample turns to pure blue from pink in the course of drying at 25°~30°C and of the minimum value of the amount of Bound Water at the point C.P. where the apparent constant weight of water in the sample is recognized in the same course of drying. The difference of the amount of water-content between those values of the maximum and the minimum amounts was recognized to agree with the amount of Bound Water which was colloidal-chemically estimated by the freezing method, solvent methods or other thermodynamic methods. This is considered correct for the reason that by both Hatschek's and Oyagi's method Bound Water having 0.8~0.7 of the value of the water-activity in the sample can be estimated, and on the other hand by the freezing method, solvent methods and other thermodynamic methods, water having almost the same water-activity value also can be estimated[1a].

Next, the present author has employed the method of estimation by electrical resistance (50 cycle resistance), and studied the relation between the amount of the water-content and the electrical resistance. The following results were obtained: With the initial decrease of the water-content in fish muscle, the electrical resistance decreased gradually. But, it began to increase rapidly in the range of 50~30% of the total water-content, which water is regarded as colloidal Bound Water; it increased more rapidly in the range of 30~20% and below 20%, which water is regarded as molecular theoretical Bound Water, the relation between the electrical resistance and the water-content showed logarithmically linear.

From the estimated value of the electrical resistance, the amount of Bound Water can comparatively easily be approximated (II).

It seems very important to examine the behaviour of Bound Water in the fish meat during the process of manufacture until it is offered as merchandise. The author has studied the subject employing the cobaltous chloride method (Oyagi's method) which can be used to estimate colloidal Bound Water, because he believes that colloidal Bound Water plays particularly important roles in the fish meat technology.

Fresh raw meat of various kinds of fish contains Bound Water of 55~82 gm per 100 gm of dried matter in maximum and 10~48 gm in minimum. When those fresh meats are naturally dried, and if Free Water only is considered to evaporate at the initial period of the drying, and at the time when the total water-content becomes 20~40% of the weight of the original matter, all the water in the dried fish meat is regarded as Bound Water. When the fresh meats of squid and Atka mackerel and other fish were dried in the room temperature, as expected, with the decrease of the total water-content in the fish meat the amount of Bound Water increased relatively; at the time when the total water-content became about 40~50%, the increase of the amount of Bound Water showed the maximum value; in the further drying, with the decrease of the total water-content, the amount of Bound Water was observed also to decrease. In this case, the binding strength of the remaining water increased with the decrease of the total water-content, and the absolute amount of Bound Water per gm of the dried matter also gradually decreased.

When the results obtained by Higuchi[12] for the meat of squid and flat fish

having various water-content by the calorimetric method and the freezing method were compared with the minimum amount of Bound Water as obtained by the present author, using Oyagi's method, both results were recognized to be agreeable. Because of such thermodynamic methods, *e.g.*, calorimetric method and freezing method are generally used for determination of Bound Water from the standpoint of colloidal chemistry, this agreement as stated above encourages the present author to believe that the minimum amount of Bound Water estimated by the cobaltous chloride method should be considered as the colloidal Bound Water.

When the salt solution is dense and the osmotic dehydrating action is promoted in the salting of fish meat, with the decreasing of Free Water accompanied with the decreasing of the total water-content, the amount of Bound Water (% in the original matter) decreased or increased relatively. In this case, some loss of the dried matter caused by dissolving out of soluble protein occur as accompanying phenomena. Therefore the change of the absolute amount of Bound Water can not be known, but according to the general examination of the results (V) which were obtained lately, it became clear that the absolute amount of Bound Water increased in the initial period of the salting, and it decreased in the final period of the salting by the denaturation of fish meat protein caused by the effect of "salting out" accompanying with the condensation of salt in muscle fluid. The increase of the amount of Bound Water in the initial period of the salting may be explained as follows: the lyotropic hydration phenomenon occurs while the amount of the salt penetrated into the sample is little and the estimating method itself gives the larger value of the amount of Bound Water and furthermore the dried matter decreases rapidly in the initial period of the salting.

When the salting solution is dilute, as a result of "salting in" effect, surface chemical adsorption of salt ion occurs rather than the osmotic action, and the absorption of abnormal amount of water was observed. Those facts are in agreement with Yamamoto's result[39)] obtained from the studies of the relation between the penetration of water through the frog's skin and the concentration of the salt solution. In the final period of the salting using the conc. solution of the salt, the phenomenon of reabsorbing water by the salt-protein complex, which has been studied by Reay[41)] and other investigators[43)], was also observed by the present atuhor. When the phenomenon which is considered to be the peptization of protein occurs, absorption of water from the outside NaCl solution is observed, and at the same time NaCl also penetrates into the fish meat muscle. At this time the amount of Bound Water was apparently observed to increase. From the compared results obtained by electrical resistance which were estimated between the fresh fish meat and salted fish meat having various amounts of water-content, a part of Bound Water having weak binding strength with fish meat protein is suggested to hydrate with electrolyte as Free Water.

During the fermentation and putrefaction of fish meat, the amount of Free Water increased while that of Bound Water decreased relatively. Those results were observed to be opposed to the results obtained in the drying or salting. It was difficult to estimate accurately the amount of Bound Water in

the fermented or putrefied meat by the cobaltous chloride method, because those meat muscle became soft and the amount of the dried matter varied in the course of the decomposition of the fish meat. Therefore the method by electrical resistance was employed for the estimation of Bound Water in such fermented or putrefied meat. According to the results obtained, when the fresh fish meat and putrefied fish meat were dried, the electrical resistance decreased with the initial decreasing of the water-content, but thereafter it increased rapidly with the decreasing of the water-content. In this case, when the putrefied fish meat was dried, it showed lower value of the electrical resistance than the dried fresh meat without putrefaction at the same water-content. From those results, it became clear that with the decomposition of fish meat muscle by putrefaction, Bound Water was weakened in its binding strength by the movement of electric charge, and the binding strength of water in the muscle tissue loosened (II).

From the previous experimental results for the dried fish meat, it was considered that in accordance with the drying, the relative amount of Bound Water increased and at last water in fish meat was only that water which is regarded as Bound Water. Therefore the author has studied the influence of the amount of Bound Water upon the velocity of drying for 12 kinds of fish meat mainly by the vapour tension method and the cobaltous chloride method. The results obtained were analyzed theoretically by B.E.T. method[23]. In this case the samples were dried at 35°~40°C in order to avoid the denaturation by heat coagulation of fish meat protein. At 35°~40°C the velocity of drying decreased remarkably in the range of the water-content of molecular theoretical Bound Water, *i.e.* the stage of drying was observed to reach apparently the 2nd stage of falling rate of drying. However, in the course of commercial drying by sun light at lower temperature, it is considered that the water-content in the fish meat reaches readily to the equilibrium in the range of the water-content of colloidal Bound Water rather than in that of molecular theoretical Bound Water. Considering from the fact that the preservability of the dried goods is owing to the lower content of water in the goods, Bound Water in the fish meat is considered to be unavailable to bacteria as a nutrient (III).

As to "Drip" in the frozen meat and fish meat, the author has studied its cause. According to the results obtained, with the falling of the freshness of fish meat, the amount of "Drip" in the quickly frozen fish meat increased gradually, when the frozen meat was stored longer, owing to the denaturation of protein. In this case it was observed that a part of water which has been estimated as "Expressible drip" in the initial period of storage has changed to "Free drip" with the lapse of storage time. As the value of differential molal free energy, $\Delta\overline{F}$, of the molecular theoretical Bound Water in the frozen and stored fish meat was less than that of fresh fish meat at the same water-content, the principal cause of the formation of "Drip" is considered to be that a part of molecular theoretical Bound Water in fish meat is set free by the dehydrating denaturation of fish meat protein (or by the denaturation owing to the condensation of salts in fish meat protein[51]).

Accepting the above consideration, if some method of increasing the value of $\Delta\overline{F}$ can be found for raw fish meat, it would seem possible to prevent to a

certain degree the decrease of value of $\varDelta\bar{F}$ during freezing and storage. Thus the good frozen foods as well as nutritiously fresh raw fish meat would be obtained.

The effect of the preventing method of the formation of "Drip" by changing of pH value of the fish meat to the alkali side (Taylor's method[34]) or the method of soaking fish meat in the NaCl solution offered by Tarr[31] were experimentally clarified to be due to the increase of the value of $\varDelta\bar{F}$ in raw fish meat before the freezing.

The remained water in fish meat after the separation of "Drip" showed a higher value of $\varDelta\bar{F}$ than that of water in frozen fish meat left as it is, therefore it seems that water after the separation of "Drip" has stronger binding power. One of the principal causes of the formation of "Drip" seems to be the denaturation of frozen fish meat protein, which denaturation brings change in the amount of Bound Water and weakens the binding power.

According to the experiment of Tarr, the higher degree of swelling of salt solution is employed, the larger the holding ability of water is, even if it is the same kind of salt, *i.e.*, the larger the preventing effect of the formation of "Drip" is. The present author has studied the swelling effect of various kinds of salt upon Atka mackerel meat, and learned that potassium salts is the most suitable substance for the prevention of the formation of "Drip" in a concentration of about 0.6 Mol (IV).

Finally, the present author has compared the autolysis of fresh raw fish meat with that of frozen fish meat. The factors concerned with autolysis were kept during the freezing storage, however the factors became less potent with the lapse of the storing time. The principal cause is owing to the denaturation of fish meat protein accompanied with the decrease of the amount of molecular theoretical Bound Water during the freezing storage.

In the glycolytic action of the fish meat, as Yamada[47] stated, with the increase of the amount of inorganic phosphorus the amount of Bound Water seems to increase. In the course of decomposition after the glycolysis, the amount of Bound Water seems to decrease. The duration time and the degree of glycolytic action are different with the kind of fish meat, therefore the factors concerned with glycolytic action during the freezing storage seemed to keep or not dependent upon the kinds of fish (VI).

VIII. SUMMARY

There are three definitions for Bound Water up to date; "biological Bound Water" from the stand-point of biology, "colloidal Bound Water" from colloidal chemistry, and "molecular theoretical Bound Water" from the molecular theory. Those definitions should be differentiated by the samples (including living matters) in various applied fields, by methods of estimation and by purposes of the studies.

In the present paper, Bound Water in the fish muscle (including mollusc and crustacean) after death was studied from the stand-points of colloidal chemistry and molecular theory.

As the method for estimating the amount of Bound Water, the author has employed (1) the cobaltous chloride methods which were offered by Hatschek at first and later on modified by Oyagi, (2) the vapour tension method, and (3) the method by electrical resistance.

As samples, the author first used the various kinds of fish and he has estimated the amount of Bound Water by the cobaltous chloride method (Oyagi's method) and by the electrical resistance method.

Next, the influence of Bound Water upon the drying velocity was studied by using 12 kinds of fish including mollusc and crustacean.

The relation between the formation of "Drip" and the amount of Bound Water during the refrigeration of fish meat was studied using Atka mackerel.

The variation of the amount of Bound Water during the process of salting of fish meat was also studied using Atka mackerel.

Last, the relation between the decomposition of fish meat protein and the amount of Bound Water during autolysis was studied.

The results obtained may be summarized as follows;

(1) Bound Water estimated by the cobaltous chloride method is to be regarded mostly as "colloidal Bound Water".

(2) The hydration of NaCl with fish meat protein is affected from the binding strength of Bound Water with fish meat protein.

(3) With the decrease of the amount of water, the electrical resistance of 50 cycles increases gradually; it increases rapidly in the range of the water-content which was regarded as "colloidal Bound Water", and it increases logarithmically in the range of the water-content which was regarded as "molecular theoretical Bound Water".

(4) On the curve of the water-content—water-activity estimated by the vapour tension method, the water which has below 0.7 of the value of the water-activity is to be regarded as "molecular theoretical Bound Water".

(5) In fresh raw fish meat, 55~82 gm of "colloidal Bound Water" is generally contained in 100 gm of the dried matter in the maximum amount, and 10~48 gm in the minimum amount.

(6) In the course of drying of fish meat, the maximum amount of Bound Water is found at the time when the total amount of the water-content in fish meat reaches to 40~50%, and the absolute amount of Bound Water decreases with the decrease of the total water-content.

(7) In the salting of fish meat, the amount of Bound Water increases apparently in the initial period of salting, and longer the salting time is, the less the amount of Bound Water is found to be. However, the amount of Bound Water apparently increases again in the final period of the salting when the fish meat protein peptizates and absorbs water. The increase of the water-content at the final period of the salting is said to be owing to the phenomenon of absorption by "salt-protein complex". In this case, when water is absorbed by fish meat from the outside solution, NaCl penetrates into the meat at the same time, and the mere apparent absorption of salt solution seems to occur.

In the case of salting by diluted NaCl solution, it may occur that the amount

of the water-content increases remarkably by the lyotropic action of electrolyte ion.

(8) With the decomposition of fish meat in fermentation or putrefaction, the electrical resistance decreases, and the amount of Bound Water decreases also.

(9) In the case of the drying of fish meat at 40°C, the drying velocity decreases remarkably in the range of the amount of water-content of fish meat which is regarded as to be contained in "molecular theoretical Bound Water". Under the natural drying conditions in the manufacture of commercial dried fish, the presence of "colloidal Bound Water" seems to inhibit remarkably the drying velocity.

(10) During the storage of frozen fish meat, "molecular theoretical Bound Water" is set free and the value of $\Delta\bar{F}$ decreases corresponding to the denaturation of the fish meat protein, which is a principal cause of the formation of "Drip".

If the increasing of the value of $\Delta\bar{F}$ is previously devised in the fish meat, it is possible to prevent the formation of "Drip". Practically the method of soaking fish meat in NaCl solution, or in solution of which the pH value is adequate, can prevent the formation of "Drip".

(11) In the glycolytic period during the autolysis of fish meat, the amount of Bound Water seems to increase and in the period of decomposition of fish meat, the amount seems to decrease.

(12) During the storage of frozen fish meat, various factors concerned with autolysis are somewhat kept as they are, but the longer the storage period of refrigeration becomes, the slower the factors act accompanying with the denaturation of fish meat protein.

LITERATURE CITED

1. (a) Akiba, M. (1951). *Bull. Fac. Fish. Hokkaido Univ.* 1 (3-4), 156.
   (b) ———— (1951). *Ibid.* 2 (3), 176.
   (c) ———— & Hirota, K. (1950). *Kagaku* ("Science") 20 (12), 572.
   (d) Hirota, K. & Akiba, M. (1951). *Bull. Res. Inst. of Applied Electricity, Hokkaido Univ.* 3 (2), 87.
2. (a) Higashi, K. (1947). *Kagaku* ("Science") 17 (5), 120.
   (b) ———— & Takeda, S. (1947). *Ibid.* 17 (3), 82.
   (c) ———— & Nukazawa, K. (1948). *Kagaku-no-Kenkyu* ("Chemical researches"), 1, Biochemistry, 23.
3. Thoenes, F. (1925). *Biochem. Zeitsch.* 157, 174.
4. Hardy, Wm. *Kolloid. Z.* 46, (1923); *Proc. Roy. Soc.* 112, 47 (1926).
5. (a) Moran, T. (1932). *Proc. Roy. Soc.* A 112, 30.
   (b) ———— (1932). *Rep. Food Inv. Board* 33.
   (c) ———— (1958). *Proc. Roy. Soc.* B 118, 548.
6. Kinoshita, K. (1930). *Jour. Chem. Soc. of Japan* 5, 261.
7. Kistler, S. S. (1936). *Jour. Am. Chem. Soc.* 58 (6), 901.
8. Plank, R. (1932). Z. ges. Kälte- Ind. 39 (4).
9. Heiss, R. (1935). *Z. Electrochem.* 41, 250.

10. Joslyn, M. A. (1940). *Ice and Refrigeration* 99 (2), 63.
11. (a) Gortner, R. A. & the Hoffman, W. F. (1922). *Botan. Gaz.* 74, 308.
(b) Newton, R. & Gortner, R. A. (1922). *Ibid.* 74, 422.
12. Higuchi, K. (1945). *Kanzume-jiho* ("The Canned Food Times") 24 (4), 8.
13. Kawakami, T. (1935). *Bull. Jap. Soc. Sci. Fish.* 4 (1), 125.
14. (a) Hatschek, E. (1936). *Trans. Faraday Soc.* 32, 787.
(b) Oyagi, Y. (1949). *Lecture of the Chem. Soc. of Japan.*
15. Katz, J. R. (1917). *Colloid-chem. Beih.* 9, 1.
16. Brooks, J. (1934). *Proc. Roy. Soc.* **B** 114, 258.
17. (a) Briggs, D. R. (1931). *Jour. Phys. Chem.* 35, 2914.
(b) ———— (1932). *Ibid.* 36, 367.
18. Herrmann, K., Gerngross, O. & Abitz, W. (1930). *Z. Chem.* 10, 371.
19. Katz, J. R. & Derksen, J. C. (1932). *Rec. Trav. Chim.* 51, 513.
20. Sponsler, O. L., Bath, J. D. & Ellis, J. W. (1940). *Jour. Phys. Chem.* 44, 996.
21. Adair, G. S. & Callow, E. H. (1930). *Jour. Gen. Physiol.* 13, 819.
22. Bull. H. B. (1944). *Jour. Am. Chem. Soc.* 66, 1499.
23. (a) Brunauer, S., Emmett, P. H. & Teller, E. (1938). *Ibid.* 60, 309.
(b) Keii, T. (1950). *Shokubai* ("Catalyst") 6, 40.
(c) Suga, T. (1950). *Kagaku-to-Kogyo* ("Chemistry and Chemical Industry") 3 (12), 387.
24. Tanikawa, E. (1943). *Enzo-Shokuhin* ("Salted Foods"). 37, Tokyo; Seikatsu-sha.
25. (a) Ueda, D. *et al.* (1935). *Denki-Hyoron* ("Journal of Electricity") 23 (4), 365.
(b) Tamura, K. (1932). *Bull. Jap. Soc. Sci. Fish.* 1 (4), 168.
(c) Yamamura, Y. (1941). *Ibid.* 10 (2), 83.
(d) Tamura, K., Miyazaki, T. & Kaziyama, I. (1933). *Suisan-Seizo-Kaishi* 1, 121.
(e) Callow, E. H. (1925). *Rep. Food Inv. Board.* 57.
(f) Yamada, K. & Kitano, F. (1948). *Bull. Jap. Soc. Sci. Fish.* 13 (6), 232.
(g) Ito, H., Kyozuka, N. & Sugizaki, C. (1949). *Jour. Agr. Chem. of Japan.* 23, 192.
26. Amano, K. & Uchiyama, H. (1948). *Bull. Jap. Soc. Sci. Fish.*, 14 (1), 48.
27. Fujiwara, S., Tsuda, K. & Yasui, S. (1913). *Jour. Imp. Fish. Inst.* 8 (8), 321.
28. Inoue, N. (1937). *Bull. Jap. Soc. Sci. Fish.* 6 (4), 187.
29. Kawakami, T. (1950). *Paper on the "Physical study on Fish Drying".*
30. Tanikawa, E. & Akiba, M. (1952). *Reizo-no-kagaku* ("Science of Cold Storage"). 248 p. Tokyo; Kōbundo.
31. Tarr, H. L. A. (1942). *Jour. Fish. Res. Bd. Canada,* 5 (5), 141.
32. *Rep of the Korean Fish. Exp. St.* (1931).
33. Moran, T. (1932). *Jour. Soc. Chem. and Ind.* 51, 20 T.
34. Taylor, G. L. (1930). *Ice and Refrigeration* 79.
35. Lloyd, D. J. *et al.* (1933). *Trans. Faraday Soc.* 29, 132; *Ibid.* 554; *Ibid.* 1228.
36. Finn, D. B. (1932). *Proc. Roy. Soc.* **B** 111, 396.
37. Samejima, M. (1952). *Gendai-Koshitsugaku-no-tenbo* 1 ("Reviews of the Modern Colloidal Chemistry"). Tokyo; Gakujutsu-tosho-shuppansha.
38. Migita, M. & Okada, M. (1952). *Bull. Jap. Soc. Sci. Fish.* 18 (3), 25; *Ibid.* 18 (4), 21.
39. Yamamoto, K. (1949). *Seitai-no-Kagaku* ("Science of Living Body") (3), 45.
40. (a) Tanikawa, E. (1943). *Enzo-Shokuhin* ("Salted Foods"). 40 p, Tokyo; Seikatsu-sha.
(b) Hasegawa, T. (1939). *Bull. Jap. Soc. Sci. Fish.* 7 (6), 339.
41. Reay, G. A. (1936). *Jour. Soc. Chem. and Ind.* 55, 309 T.
42. Rusznyák, S. (1921). *Biochem. Z.* 114, 23.
43. Callow, E. H. (1932). *Rep. Food Inv. Board* 101.
44. Yamada, K. (1948). *Bull. Jap. Soc. Sci. Fish.* 13 (4), 113.
45. Kimoto, E. *et al.* (1951). *Igaku-to-Seibutsugaku* ("Medicine and Biology") 20 (3), 110.

46. Amano, K. (1953). *Bull. Jap. Soc. Sci. Fish.* 18 (10), 164.
47. Yamada, K. (1952). *Koso* ("Enzyme"). 345 p, Editted by Sakaguchi, K. and Asai, Y., Tokyo; Asakurashoten.
48. Yamada, K. (1948). *Bull. Jap. Soc. Sci. Fish.* 14 (1), 23.
49. Kimata, M. (1941). *Jour. Imp. Fish. Inst.* 34 (2), 116.
50. Kuprianoff, J. (1958). *Soc. Chem. Ind.* 14.
51. Simidu, W. & Hibiki, S. (1952). *Bull. Jap. Soc. Sci. Fish.* 17 (10), 13.

STUDIES ON THE STRUCTURE AND GROWTH OF THE SCALES IN THE GOLDFISH

JURO YAMADA

STUDIES ON THE STRUCTURE AND GROWTH OF THE SCALES IN THE GOLDFISH

JURO YAMADA

Faculty of Fisheries, Hokkaido University, Hakodate, Japan

CONTENTS

I. INTRODUCTION

The method of age determination and estimation of life history by means of fish scales has been widely used in population studies of teleost fish since the beginning of this century, when Hoffbauer observed the concentric rings on scales of the carp and thought them to be an indicator of their age. So far, studies on fish scales had been mainly concerned with their phylogenetic and taxonomic meaning but not with their ecological value.

The theory offered by Hoffbauer is based on the assumption that a change in the growth rate of a fish would cause the distance between contiguous ridges on the scales to vary and there would cause an annual ring or a growing zone to form. Many investigations into the determination of the age of fish have been carried out on various species followed this theory, but insufficient fundamental knowledge on the scales has sometimes led workers to erroneous conclusions, because the method was rather empirical but not intimately connected with the mechanism of the development or of the growth of the scale structure. Many arguments have been indulged in, therefore, on the reliability of this method.

In this connection, it was supposed that detailed studies on the morphology of each constituent of the scale structure and on the physiology of the scale

growth would make this method more accurate and would improve the population study of fish as well. This is the reason why the author undertook a comprehensive study of the scales of goldfish (*Carassius auratus L.*). The goldfish was chosen primarily because of the facility in rearing; its growth is rather rapid and the larvae are obtainable with ease. The second reason is its possession of typical cycloid scales which are provided with many concentric ridges and several radial grooves. As the general form and the orientation of the scales are supposed to be influenced by the form of the fish body, the type of the goldfish used in this study is exclusively "Wakin" which is closely similar to the crucian carp in form.

The present paper includes morphological observations on the development, regeneration, and absorption of the scales, and also discussion of some physiological aspects of these phenomena. A teleost scale is formed in the mesodermal connective tissue beneath the epithelium, differing from a placoid scale of selachians which develops as one of the integumental structures such as tooth, spine and nail etc. of ectodermal origin. Accordingly, the studies reported in this paper have been carried out under the assumption that both the morphology and the physiology of the formation or growth of the goldfish scales are much the same as those of a membrane bone. The development, regeneration and absorption of the scales are explained in osteological terms; histochemical tests on alkaline phosphatase, polysaccharides and others, and an injection experiment of parathyroid hormone are also employed, because these substances are supposed to be taking part in the formation and the ossification of the scales. Besides these, some experiments, including the transplantation of scales and the re-examination of once removed scales which were returned into the skin after pictures had been taken, were designed to see the change of scale structure which occurred on the same scales at different stages after a certain period of rearing of the fish.

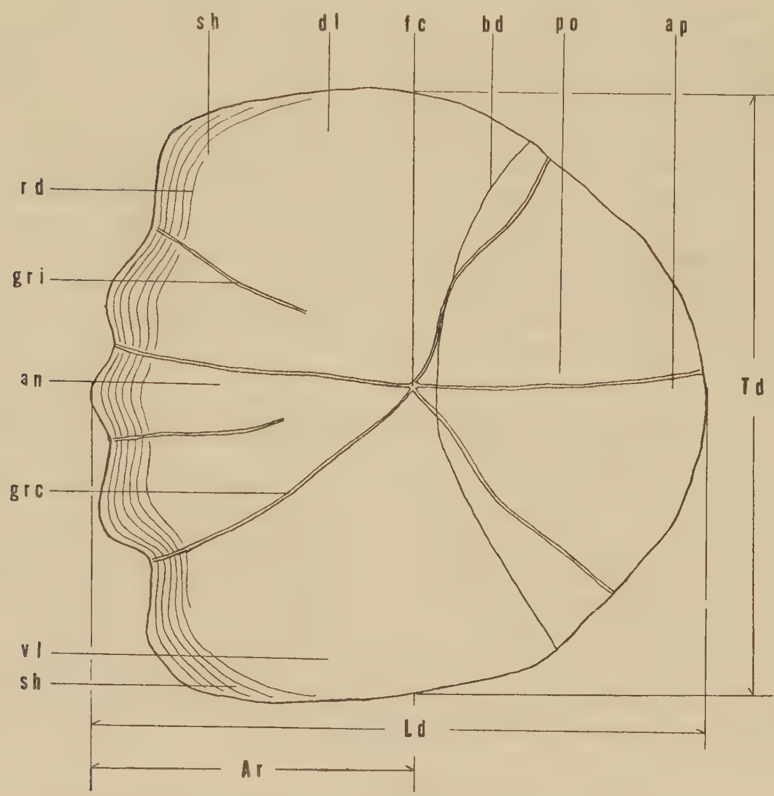
The methods employed in the present study will be described fully in each section.

Before going further, the author wishes to express his sincere gratitude to Professor Saburo Saito of the Faculty of Fisheries, Hokkaido University, for his kindness in directing and encouraging the author throughout the whole course of this work. He is also greatly indebted to Emeritus Professor Tetsuo Inukai, Hokkaido University, for his invaluable suggestions and kind criticism while this paper was in preparation.

II. DEFINITIONS

Clear definitions of the structures and parts which constitute the whole scale are necessary in order to avoid possible confusion because too many names have been employed variously by different authors. In this paper, the following terms will be adopted partly on the recommendation of Kobayashi (1958) (Text-fig. 1).

Anterior part of scale: this is the part anterior to the focus, provided with closely set ridges; called also the "covered" or "unexposed" part, but these



Text-fig. 1. Diagrammatic drawing of a typical goldfish scale
Ar: anterior radius; Ld: longitudinal diameter; Td: transverse diameter; an: anterior part of scale; ap: apex; bd: border between exposed and unexposed parts; dl: dorso-lateral part of scale; fc: focus; grc: complete groove; gri: incomplete groove; po: posterior part of scale; rd: ridge; sh: shoulder; vl: ventro-lateral part of scale

names do not strictly mean the anterior part of scale because the most part of scale is covered with surrounding scales.

Posterior part of scale: the side opposite to the anterior part, provided with rather sparse ridges; the name "exposed" part is also used in the sense described above.

Lateral parts of scale: the upper and lower sides of scale, called respectively dorso-lateral or upper lateral and ventro-lateral or lower lateral.

Shoulders: the two anterior corners of both the lateral sides.

Apex: the posterior-most end of the exposed part.

Focus: variously called nucleus, center or centrum; this is not the geometrical center of the scale but it is the center of its growth.

Ridges: linear elevations of the bony layer of the scale; the term "circuli" or "striae" is most often used. But "ridges" is more suitable because this feature does not necessarily possess concentric circular lines.

Grooves: known by the name "radii" for the reason that these structures appear radially from the focus. Those which start from the focus and reach up to the scale margin are called "complete grooves", whereas others which do not start from the focus or do not reach to the margin are "incomplete grooves".

Longitudinal diameter: the length of the median line passing through the focus and the apex. As the anterior margin shows some jaggedness in large scales, a certain amount of error in measuring the diameter is unavoidable.

Transverse diameter: the length of the line which is drawn at right angles to the longitudinal median line and crosses with it at the focus.

Anterior radius: the anterior half of the longitudinal diameter, limited from focus to anterior margin.

III. GENERAL DESCRIPTION OF THE SCALE PATTERN

Scales of the goldfish vary in form and in superficial structure with the different body parts and with the different body size of the fish. The main part of the body is covered with pentagonal scales which are considered to be standard in form. There are about thirty scales forming the lateral line, above and below which are respectively six and seven rows of scales. It is believed that for one to observe all these scales covering one side of the body surface of individual fish of different body size would be useful in mastering the general features of the scale pattern and in considering the essential nature of the structures which constitute the scale pattern.

Procedure

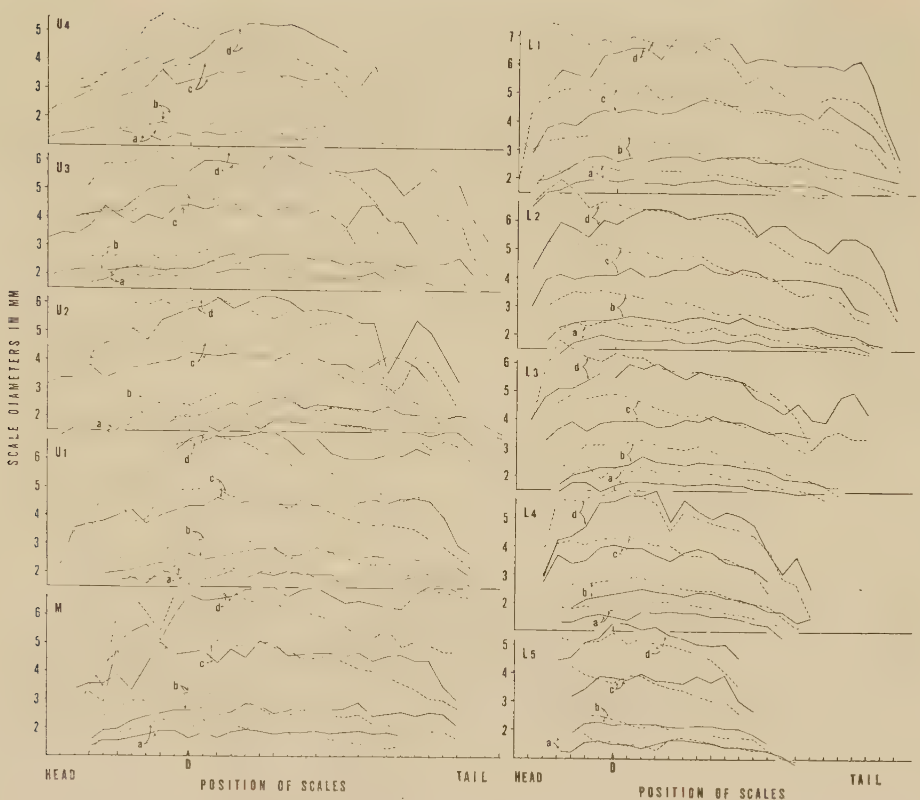
Nearly all scales covering the left side of the body were pulled out from five specimens. Those of the uppermost and lowermost scale rows were omitted because of their smallness and of their irregular form. The fish from which the scales were taken measured respectively 3.8, 5.2, 7.8, 9.8 and 11.3 cm in body length. The scales were arranged on glass slides in regular sequence according to their arrangement on the body surface. Measurements were made on these scales, magnified by means of a shadowgraph, of their longitudinal diameter, transverse diameter, anterior radius, and number of ridges and grooves.

In each individual fish, there are always a considerable number of regenerated scales which can be distinguished easily from ontogenetic ones by their rather numerous radial grooves and by an irregularly formed network of the focal area in which are found no concentric ridges. Further, there are a few extraordinary scales located at the extremities of both anterior and posterior ends of each scale row. The anteriorly located scales are strongly compressed along the longitudinal axis, while the posterior ones are along the transverse. In treating the obtained data, the exception of the above noted uncommon scales was taken into consideration as occasion demanded.

Observations

(1) Longitudinal and transverse diameters

The anteroposterior trends of the two scale diameters are given in Text-

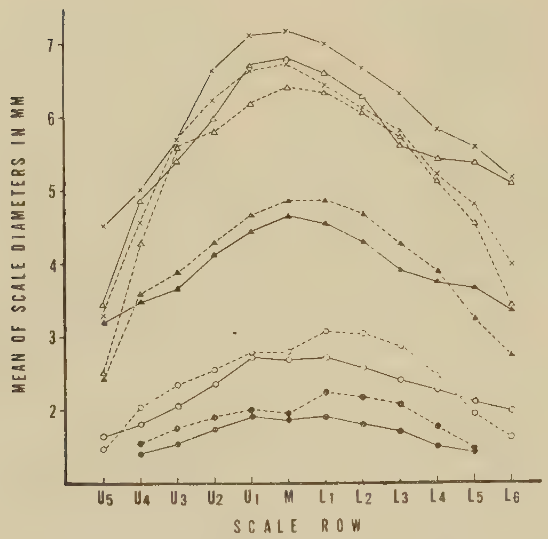


Text-fig. 2. Anteroposterior trend in change of two scale diameters of all the scales in every scale row with four specimens
D: position of the origin of dorsal fin base; U_1: upper first scale row; M: lateral line scale row; L_2: lower second scale row
The specimens are: a, 3.8 cm; b, 5.2 cm; c, 7.8 cm and d, 9.8 cm in body length.
——— longitudinal diameter - - - - - transverse diameter

fig. 2. Since the number of scales in a given scale row varies individually, the origin of the dorsal fin base (D) was adopted as the standard.

Generally speaking, the transverse diameter is larger than the longitudinal in the anterior part of the body, while in the posterior the relation is reversed. This is clearer in the lower (ventral) half of the body than in the upper (dorsal). The maximum longitudinal diameter is within the part below the dorsal fin while that of the transverse appears more anteriorly. There is at least one point in every row where the two diameters come to be equal. In front of this point, the width of scale is larger than the length while the latter gets ahead of the former behind this point. These points appear to have a tendency to move forward as the fish grow. In other words, the scales grow longitudinally rather than transversally in accordance with the growth of their bearer (see also Text-fig. 3).

In order to compare the scale size amongst the individual rows, the mean



Text-fig. 3. Mean of two scale diameters of scales existing in the space between the origin of dorsal fin and that of anal fin with every scale rows with five specimens

——— longitudinal diameter
------ transverse diameter

● 3.8 cm in body length
○ 5.2 〃
▲ 7.8 〃
△ 9.8 〃
× 11.3 〃

of the scale diameters for every scale row was calculated (Text-fig. 3). In this case, because of the fact that the deformed scales in both the anterior and the posterior body parts vary largely in their diameters (Text-fig. 2), only the scales existing in the space between the origin of the dorsal fin and that of the anal fin were used in order to make the comparison accurate. The two diameters, either longitudinal or transverse, show a higher value in the upper first (U_1), lateral line (M) and lower first (L_1) than in other scale rows. In the smaller fish (3.8 and 5.2 cm), the mode of the longitudinal diameter is at U_1, whereas that of the transverse is at L_1. But in the larger fish (7.8, 9.8 and 11.3 cm), the modes, both of the longitudinal and transverse, move towards M. At the same time, it must be noticed that the two diameters are different from each other in growth rate, so the longitudinal which is shorter in the smaller fish tends to surpass the transverse as the fish grows.

(2) *Position of focus*

As the focus is the growth center of a scale, its position in the scale shows the growth rate of each scale-part after the origin of the scale has been established. In this study, the position of the focus in the longitudinal axis of a scale was represented as the ratio of the anterior radius to the longitudinal diameter. A value above 0.50 means that the focus is located posterior to the middle of the longitudinal axis.

Table 1. Frequency of scales of a specimen, 7.8 cm in body length, grouped by series of the ratio of anterior radius to longitudinal diameter, to show the transition of the position of focus in one individual fish

Scale row	Anterior radius / Longitudinal diameter						Total number of scales except the regenerated
	less than 0.35	0.36 ~0.40	0.41 ~0.45	0.46 ~0.50	0.51 ~0.55	more than 0.56	
U_5	1	2	11	6	0	0	20
U_4	2	3	10	7	1	0	23
U_3	1	4	10	10	1	0	26
U_2	2	1	6	10	6	0	25
U_1	2	1	3	9	13	2	30
M	1	0	4	4	17	1	27
L_1	1	1	2	6	14	1	25
L_2	1	1	2	11	9	0	24
L_3	2	1	0	12	5	0	20
L_4	2	1	3	10	1	0	17
L_5	1	5	8	0	0	0	14
L_6	3	8	2	0	0	0	13

First, to see the relative change of this ratio in one specimen, the calculation was made of all scales except the regenerated ones in a specimen of 7.8 cm body length. Table 1 shows the frequency of the scales in several grades of the ratio with every scale row. It is quite clear that the foci of the scales of the lateral line row tend to lie posterior compared with those of the scales of the other rows, and that the tendency decreases, in turn, as the row goes dorsal- as well as ventral-ward. Then, the position of focus was compared individually, in the same way, with the scales of four rows (U_2, U_1, L_1 and L_2). Lateral line scales were omitted in this case because in the smaller fish it was difficult to decide exactly the position of the focus owing to the presence of the opening of the lateral organ. It can be seen in Table 2 that the foci of the scales tend to change their position backward with the growth of the fish body.

These facts reveal that the growth rate of the anterior part of scale exceeds slightly that of the posterior as the scales grow larger. That the lateral line

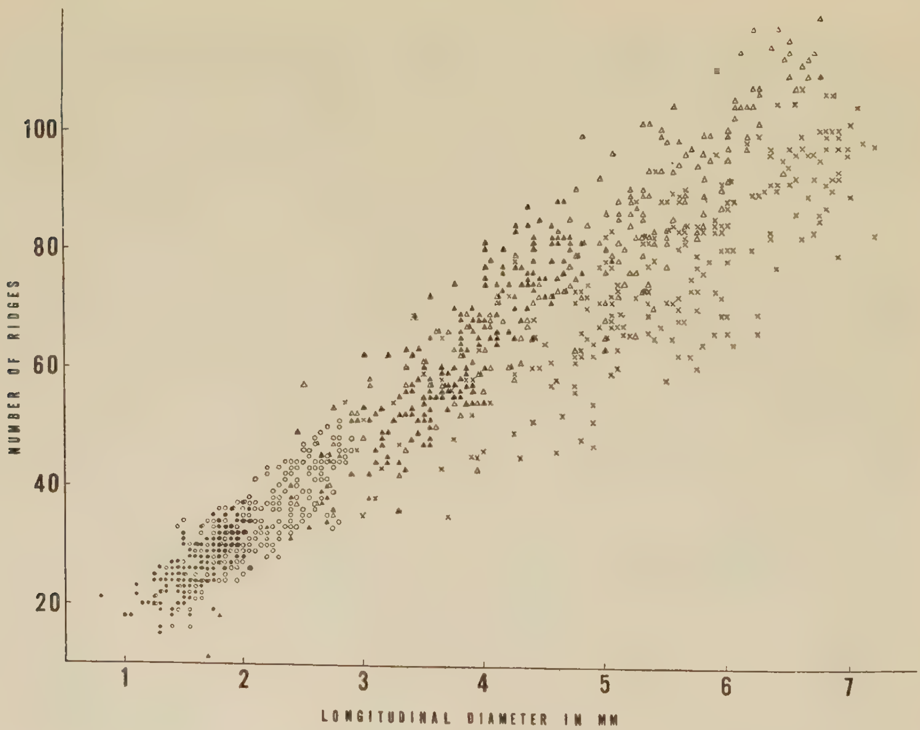
Table 2. Frequency of scales in four scale rows (U_2, U_1, L_1, and L_2) of five specimens grouped according to ratio of anterior radius to longitudinal diameter, to show the transition of the position of focus accompanying the fish growth

Body length (cm)	Anterior radius / Longitudinal diameter						Total number of scales except the regenerated
	less than 0.35	0.36 ~0.40	0.41 ~0.45	0.46 ~0.50	0.51 ~0.55	more than 0.56	
3.8	15	40	33	4	0	0	92
5.2	11	27	50	15	1	0	104
7.8	6	4	13	36	42	3	104
9.8	3	2	6	20	43	5	79
11.3	6	4	17	50	19	0	96

scales show the highest ratio may be concerned with the fact that these scales are the largest in longitudinal diameter among all scales. It should not be considered, however, that the focus appears originally in the real center of the scale when the first ridge is formed, otherwise, it would follow that a ratio less than 0.50 would indicate a higher growth rate in the posterior part of a scale rather than in the anterior at the early stage of growth.

(3) *Number of ridges and their intervals*

As a scale grows larger, ridges appear one by one on the upper surface of scale as linear elevations of bony substance. They increase in number in proportion to the growth of the scale (Text-fig. 4). There exists a certain extent of variation between the number of ridges and the scale size, that is to say, the intervals between ridges are variable in different scales. Since the alteration of ridge intervals is useful in age determination, the number of ridges should be considered with reference to their intervals. In view of this point, it is more reasonable to compare the number of ridges with the anterior radius rather than with the longitudinal diameter. The value obtainable in dividing

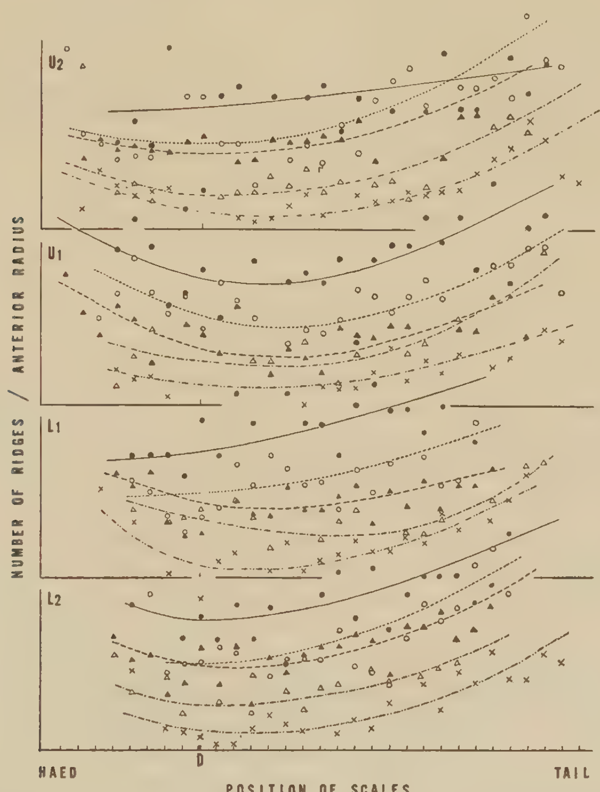


Text-fig. 4. Correlation between number of ridges and longitudinal diameter in all scales of five specimens

● 3.8 cm in body length △ 9.8 cm in body length
○ 5.2 〃 × 11.3 〃
▲ 7.8 〃

the number of ridges by the anterior radius represents the density of ridges or the average distance between two adjacent ridges on one scale.

Of all scales in the four rows excepting the regenerated scales, the value was calculated and arranged anteroposteriorly with five specimens (Text-fig. 5). The scales show a large variation in this value with each other. But, on the whole, it may be said, though roughly, that with every row there is a tendency of a sharp decrease in the value in the anterior body part and a gradual increase towards the caudal part. No difference among the four scale rows could be found. Moreover, one can see in every scale row a gradual lowering of these values according to the growth of the fish.



Text-fig. 5. Anteroposterior trend in change of the density of ridges (number of ridge/anterier radius) with four scale rows with five specimens, except the regenerated scales

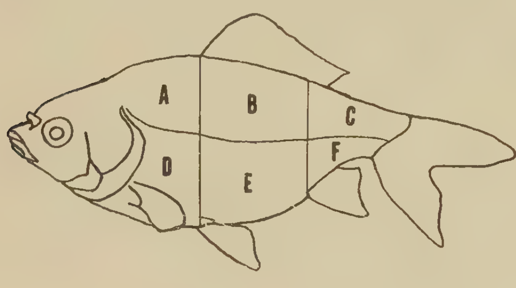
——●—— 3.8 cm in body length　　·—·—△—·—· 9.8 cm in body length
······○······ 5.2 〃　　———×——— 11.3 〃
——▲—— 7.8 〃

From the above facts, two conclusions can be drawn: 1) The density of ridges varies with the body part, viz., apart from the foremost few scales which are strongly compressed along the body axis, the relative number of ridges is

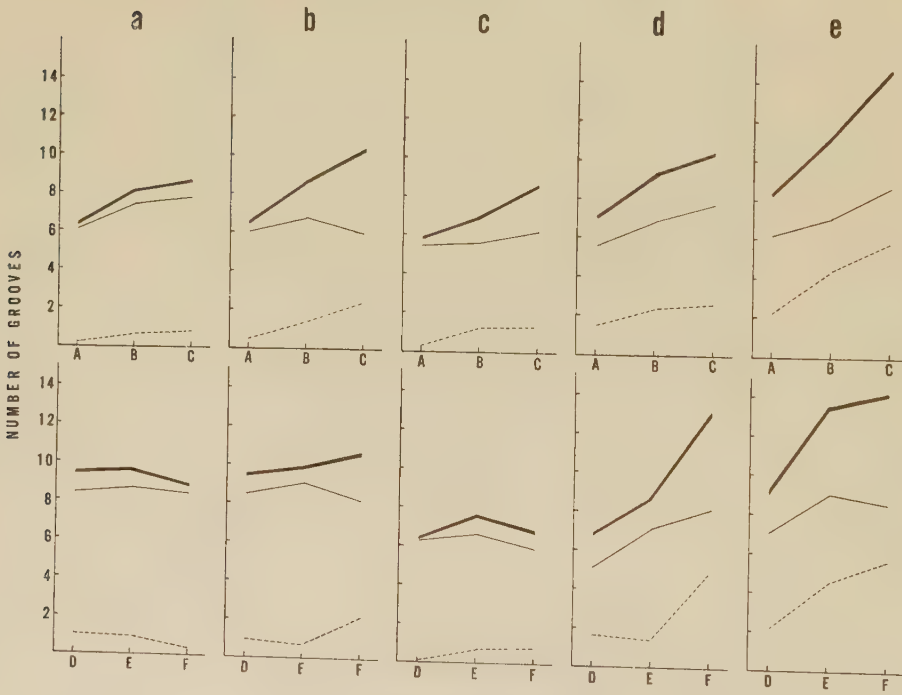
small in the anterior scales whereas there are abundant ridges in those of the caudal part in proportion to their short radius. 2) The number of ridges in a certain width of a scale decreases with the enlargement of the scale, so the intervals between ridges grow larger according to the degree of fish growth. The facts seem to suggest the manner of ridge formation.

(4) *Number of grooves*

In each scale, there are several grooves running radially from the focus to



Text-fig. 6. Showing six parts of body surface divided for comparison of average number of grooves with the scales in each part. The lateral line scales are included in the upper three parts.



Text-fig. 7. Comparison of average number of grooves with the scales in six parts illustrated in Text-fig. 6

a: 3.8 cm in body length
b: 5.2 〃
c: 7.8 〃
d: 9.8 〃
e: 11.3 〃

—— total number of grooves
—— complete grooves
---- incomplete grooves

the periphery. They can be called "complete grooves" in contrast to "incomplete grooves" which do not start from the focus or do not reach to the edge. Almost all of the incomplete grooves are those which do not start from the focus. Various theories as to the function of the grooves have been proposed by many authors. The predominant opinion is that they give flexibility to the scales when a fish is in the act of bending the body (Taylor, 1916; Neave, 1940; Wallin, 1957). As a proof of this opinion, Taylor and Wallin showed in the pigfish and the roach, respectively, a gradual increase in number of grooves from the inflexible head part to the most flexible caudal part. In this connection, the relation between the number of grooves and different body parts was also studied in the goldfish. The body was divided into six parts as shown in Text-fig. 6. The average number of both complete and incomplete grooves of the scales within each part, excepting the regenerated scales, is shown in Text-fig. 7. On the whole, the grooves including both the complete and the incomplete have a tendency to increase in number from the head part to the caudal part. They also increase in number with the growth of the body. The figure seems to show that this increase in total number of grooves in parts from head to caudal or with the growth of body should be considered due mainly to the increment of the incomplete grooves and not of the complete.

Consideration

The observations made in this study concerning the change in general shape of the scales with the different body parts and with the different body size can be summarized as follows. 1) The scales in the anterior body part have longer transverse diameter than longitudinal, whereas in the positions following posteriorly they come to show longer longitudinal diameter than transverse. 2) The longest longitudinal appears at a point in the space below the dorsal fin, but the transverse appears more anteriorly. 3) In comparison between the scale rows, the longest longitudinal is shown in the scales of the upper first row in the smaller fish, while the longest transverse is in those of the lower first. In the larger fish, on the other hand, the maxima of both the longitudinal and the transverse appear in the lateral line scales. 4) The rate of the longitudinal growth of the scales is larger than that of the transverse growth.

There is almost no report concerning change in the scale pattern with different body parts of a fish. Only Saito (1956) worked on all the scales of both sides of the body of the crucian carp; he reached conclusions most of which are in accord with the present results obtained in the goldfish. It is quite clear that the general form of the scales is strictly affected by the body-part where they grow. When the body is enlarged with the growth of the fish, the scales covering the body surface would grow in size. Accordingly, the difference in growth rate on a certain body-part may lead to the difference in form of the scales. The fact that the longitudinal diameter of scales tends to surpass the transverse with the growth of the fish shows that the fish grows longitudinally more than transversally. As the relative volume of the viscera is considered to be large in proportion to the whole body in the smaller fish, the bulge of the belly is supposed to cause the comparatively longer transverse diameter in

the lower scale rows in these fish.

The change in position of the focus and in number of ridges should be considered together with the aforementioned facts. The position of the focus moves posteriorly as the scales grow, in other words, the forward growth of the scales exceeds the backward. Moreover, in looking over the whole group of scales of one individual, it is clear that the large ones such as those in the lateral line, except some extremely anterior ones which have been prevented from forward growth by the clavicle, possess a posteriorly placed focus. As a rule, the larger the scale the more ridges there are, but the number of ridges is not strictly in proportion to the anterior radius even on one individual fish. The density of ridges is less in the large scales of the middle body-part which show the comparatively longer anterior radius than in those of the anterior or of the caudal part. The comparison of Text-fig. 2 with Text-fig. 5 will make clear the relation between the transition of the longitudinal diameter of scales and that of the density of ridges. The two are roughly in inverse relation; the body-part where the longitudinal diameter shows its maximum corresponds with the minimum density of ridges. The finding may be interpreted that the ridges formed at a certain length of radius are less in number in the scale which has grown at a higher rate. This idea seems to indicate the significance of the growth rate of scale in respect to the density of ridges.

The number of grooves grows larger anteroposteriorly within the same individual; it also increases all over the body with the growth of the fish. The increment takes place by the appearance of new incomplete grooves. The facts alone are insufficient, however, to lead to a conclusion that the grooves play a role giving flexibility to the scales as Taylor and Neave insists; this will be further discussed on the basis of observations on the structure of grooves.

IV. DEVELOPMENT OF SCALES

Studies on the development of fish scales form the most essential part of the histology and the morphology of growing scales. For this reason, it has long been one of the main subjects of many investigators who have taken a great interest in fish scales. Among many investigations into the histogenesis of scales in various fishes, the early development of scales in the goldfish was studied by Neave (1940), also Pevsner (1926) worked on the crucian carp, *Carassius vulgaris*. Regardless of species used in the studies of this subject, there are some unsolved questions and disagreements in views. The present author himself once studied the development of the scales in the rainbow trout (Yamada and Saito, 1952), and now he admits the necessity to observe it again in the goldfish.

Procedure

The larvae used in this study were obtained from a pond in the campus of the Faculty of Fisheries. They grew about 15 mm in body length within a month after hatching, specimens of the larvae were fixed weekly in Bouin's solution, sectioned by the paraffin embedding method and stained with Delafield's haematoxylin and eosin.

Observation

The first indication of the origin of scale appears as an aggregation of cells which is called "scale papilla" or "cutis papilla." Prior to the appearance of this scale papilla, the skin consists simply of two layers, the outer stratified epithelium and the inner fibrillated corium, between which no tissue layer can be observed (Fig. 1). When the fish grows as large as about 12 mm in body length, there appears a thin layer of connective tissue cells, some of them aggregate in many evenly spread masses at regular intervals to form scale papillae (Fig. 2). The observation of a series of sections showed that the papillae are formed first in the anterior body-part along the presumptive lateral line; thereafter, they rapidly spread over the body surface both longitudinally and transversally.

The cells transformed into the scale papillae are basophilous and larger in size, more rounded in shape than the original connective tissue cells; within each is contained an oval nucleus. The aggregation of these cells comes to be divided into two layers, the upper and the lower, by the appearance of a fine horizontal crack at the middle of the mass (Fig. 3). The cells become thinner losing their cytoplasm and are transformed into spindle-shaped cells lying parallel to the crack. Now, in the crack, a thin homogeneous light-reflective layer appears (Fig. 4). This is the origin of a scale, exactly speaking, the origin of the upper bony layer of a scale. Accordingly, the cells in the papillae should be called "osteoblasts" different to the original connective tissue cells which may be regarded as "fibroblasts." The osteoblasts around the origin of a scale are flattened more and more while losing their cytoplasm, and they finally begin to disappear passing through the stage of naked nuclei which adhered closely to their own product. The degeneration of osteoblasts begins from the central region of the upper side of the just-formed scale (Fig. 5). Thus established scales continue to grow larger while passing through the above described stages pushing down the corium at the anterior and pushing up the epithelium at the posterior, so their growth results in an imbricated oblique arrangement of the scales. The connective tissue left between two scale papillae becomes fibrous and elongates obliquely making itself into a thin fibrous wall which borders two adjacent scales connecting the epithelium to the corium. In this way, each new-born scale comes to be enclosed separately by the connective tissue wall which is called the "scale pocket."

The osteoblasts degenerate completely at the upper surface of the scale except the growing margin, while they remain as a layer at the lower surface and connect to the upper cells around the scale margin which is covered with one or two layers of them (Fig. 6). The lower layer cells also disappear finally, so the lower surface of the scale comes to be in direct contact with the underlying wall of the scale pocket. After this stage, the osteoblasts are seen only at the scale margin; they are contributing to the growth of the scale. Meanwhile, another layer of the scale, the fibrillary plate, is formed beneath the bony layer (Fig. 7). It consists of several layers of fibrous lamellae which are added to the scale one by one with the enlargement of the bony layer. Thus, it is under-

stood that the scale is composed of two different layers, the upper bony layer and the lower fibrillary plate; the former grows horizontally in almost definite thickness while the latter grows both horizontally and vertically by adding successively larger new lamellae.

The ridges, linear concentric rings in upper view, are observed as spicular projections of the bony layer in section (Fig. 7). The tips of the projections invariably lean toward the scale focus. The first ridge appears as early as the time when the lower layer cells still remain at the lower surface of the scale (Fig. 6). There is no special structure which seems to be forming the ridges, but it appears that the ridges are formed one by one as a result of accumulation of bony material which filled the intercellular space between two osteoblasts in contact with the growing ridges. The inner cell subsists still after the degeneration of the outer cell until the outer two or three new ridges develop. The ridge is low and dull when it arises at the outermost periphery of the scale; it gradually increases in height until it finally attains its ordinary height of about 5 μ and its ordinary steepness while two or three new ridges are at the outside. There are often seen naked nuclei attached at the inside of the inner ridges. These facts imply that the inner cells remain at the inside of the just formed ridge and contribute to the growth of the ridges in the consumption of their cytoplasm.

The grooves in section appear as a partial absence of the bony layer (Figs. 8 and 9). The edges of the trough-shaped section show somewhat elevated bony material. Fibrous lamellae of the fibrillary plate become loosened and wave appreciably just under the grooves. There is usually a polygonal cell which entered into the peripheral part of a groove (Fig. 8), but no cellular element can be seen in the groove when the sections passing through the inner part of a scale are observed (Fig. 10; cf. Fig. 24).

In the cross sections of a specimen, 14 mm in body length, it was observed that the scales of five middle scale rows including the lateral line, developed fully along the body axis from anterior to posterior, whereas those of the upper and the lower third rows, just outside of the above mentioned rows, were in the stage of scale papilla. In a fish two months from hatching, 21 mm in body length, the scales had developed fully covering the whole body surface.

Discussion

So far as the author knows, there is no datum concerning the body size of the goldfish in which the scale papillae appear first. The time after hatching or the body size when the scales come to develop cannot be decided exactly because consideration must be given to the fact that the growth rate of young fish is greatly affected by the environmental conditions in which they live; however, the present study showed that the scale papillae do not appear until the fish grows to about 12 mm in body length or until a month has passed after hatching. The papillae are formed first along the lateral line in the anterior half of the body. It has been confirmed by many investigators that the scales in various fishes first appear in the neighbourhood of the lateral line. Particularly, Klaatsch (1890), Hase (1911) and Neave (1936) found respectively in the brown trout,

the perch and the kamloops trout, that the scale formation starts from the anterior and middle region of the body; on the other hand, Paget (1920) as well as Yamada and Saito (1952) showed the first appearance of the papilla in the posterior half in the brown trout and the rainbow trout. The site where the first papillae are formed might be different with the species. But it seems to be sure of all species that they appear first in the neighbourhood of the lateral line and last in the back and belly.

Klaatsch (1890), who first made detailed phylogenetic study of scales from the placoid of selachians to the cycloid of teleosts, pointed out that the scales of the trout are mesodermal in origin and that they consist of two different layers, bone and hardened connective tissue. There have occurred heated controversies related to the dermal origin of teleost scales since the end of the last century. Fach (1937) seems, however, to be the last investigator to insist on the participation of epithelial cells in the formation or growth of the scales. Today, nobody would doubt the fact that the teleost scales develop as one of the completely mesodermal organs. But, concerning the genesis and nature of two constituents of the scale, the bony layer and the fibrillary plate, there are several essential problems still to be solved.

Among many workers who have studied the histogenesis of fish scales, Neave (1936) introduced the osteological concept into the study of the development of the trout scale suggesting that the stages in the laying down of the bony layer are typical of the formation of membrane bone which originates directly in the connective tissue. The present author proved the propriety of Neave's opinion in his previous paper (1956) by applying histochemical tests to the developmental stages of the rainbow trout scale. The process should be essentially the same regardless of the species used, but, so far as the present author has studied, the goldfish seemed to be not as good material as the rainbow trout for observation of the developmental stages of the scales, because the goldfish scales even in germ stage are not soft enough to make good thin slices and further because of the inconspicuousness of the original papilla. But the process could be traced almost satisfactorily from the osteological point of view comparing it with the process observed in the scales of the rainbow trout.

Before the appearance of the scale papilla, the corium is composed of coarse fibre bundles in which few or no cellular elements can be seen. Neave showed in his clear figures that the formation of scale papillae in the trout skin is not caused by the increase of corium cells *in situ* but is based upon the outgrowth of mesenchymes arising at the lateral line region. This may be true of the goldfish because no multiplication phase of fibroblasts was observed in the corium. The scale papilla of the goldfish takes a more depressed form and is larger in size than that of the rainbow trout. The scale itself, therefore, is also larger in size when it comes visible in the papilla. There is no doubt that at first the origin of a scale consists of only the bony or upper layer because the ridges are formed later on it, though Pevsner (1926) in *Carassius* and *Rutilus* or Petrov and Petruschewsky (1929) in *Cyprinus* maintain that the fibrillary plate arises prior to the bony layer.

Concerning the genesis of the bony layer and the fibrillary plate, there are

various opinions expressed by a number of investigators which may be divided roughly into three categories, though each of the investigators has his own shade of difference in meaning. 1) The bony layer is laid down by the upper layer cells of the papilla while the fibrillary plate is formed at the expense of the lower elements (Ussow*, 1897; Nusbaum, 1907; Paget, 1920; Pevsner, 1926). 2) The original cells which form the scale papilla contribute merely to the formation of the upper layer of scale and the fibrillary plate is derived from the underlying connective tissue wall (Klaatsch, 1890; Hase, 1911; Neave, 1936, 1940; Yamada and Saito, 1952; Dietrich, 1953; Yamada, 1956). 3) Opinions which do not fall within the above two. Setna** (1934) is of the opinion that the lower layer cells give rise to the upper cells and on turning around the scale margin each cell stops forming fibres and begins to secrete bony material. On the contrary, Creaser** (1926) supposes the cell migration from the upper to the lower side. Wallin (1957) opposes earlier views; he shows that both the layers are derived from the same germ structure at the periphery and that the bone is transformed to the fibrillary plate. These authors admit the formation of different materials by the same cells.

As is above stated, what is first formed in the papilla is the bony layer and not the fibrillary plate. This structure appears horizontally in the middle of the cell aggregation in the same way as in the formation of membrane bone which takes place in the aggregation of osteoblasts. The theory that the bony layer is the product of only the upper cells seems to be based upon the fact that the lower cells continue to remain in the lower surface of a scale after the degeneration of the upper cells has completed excepting only at the scale margin. Since these lower cells also disappear finally, difficulty arises in interpreting the thickening of the fibrillary plate all over the surface after the disappearance of lower cells, if they act really in forming fibres without taking part in laying down of bone. If that is not the case, the fibrillary plate must be naturally formed by cells of the underlying connective tissue of the scale pocket wall. It was clearly observed in the rainbow trout, however, that the fibrillary plate as well as the bony layer is enclosed by a sheath of osteoblasts at the scale margin; this leads to the impossible thought that the fibrous lamellae must be added through these interposed osteoblasts.

Therefore, the present writer could not help confessing the complicacy of the problem. Recently, however, he noticed that some of the photomicrographs in the previous paper (1952) contain matter useful for the solution of this problem. The older figures are shown again for reference (Figs. 10, 11). In the figures the latest fibrous lamella can be seen in course of formation; it has a connection with the marginal osteoblasts at both the anterior and the posterior margin of the scale. But, it is impossible to consider that the osteoblasts themselves are being transformed to the lamella at the centre of the scale after they have formed bony material at the margin. The following may be more reasonable explanation. The pocket wall provides the scale with a cellular sheath which covers the scale except its upper surface, and at the next stage the sheath

* Cited from Nusbaum (1907). ** Cited from Neave (1940).

turns into osteoblasts at the scale margin and fibrous lamella at the lower surface of the scale. Fibroblasts of the connective tissue, which will be transformed into osteoblasts at the scale margin where there is no scale on them, form or are transformed into fibrous lamella at the lower surface of the scale probably being influenced by the presence of the scale itself. Moreover, the addition of a new fibrous lamella is always preceded by the extension of the bony layer. At the beginning of the scale formation, the papilla is filled with osteoblasts which produce only the bony layer. After the formation of bony material has been completed the lower layer cells degenerate turning into fibrous lamella. The situation is kept at the scale margin as long as the scale grows larger, while at the inner part of the lower surface the fibroblasts produce fibres without passing through the stage of osteoblasts. In this way, the cellular sheath around the scale comes to be composed of marginal osteoblasts and lower fibrous lamella. It is natural, therefore, that an intimate relation exists between the extension of bony layer and the addition of fibrous lamellae. For instance, Paget found in the scales of *Salmo fario* that the thickness of a fibrous lamella varies according to the distance of ridges which correspond to the edge of the lamella. Also Lindahl and Wallin (1955) have shown in the roach scale that in most cases one fibrous lamella is added for every two ridges of bone.

The above interpretation can be applied to the regeneration of scale. When a scale is removed from the scale pocket, the regenerated scale appears in it within a very short period. As the marginal osteoblasts are supposed to be removed together with the scale, the rapid regeneration must be made by the cells proliferated from the pocket wall which normally produce marginal osteoblasts and fibrous lamellae. Nevertheless, they actually produdce first the bony layer followed next by the addition of fibrous lamellae.

The process of ridge formation raises a very important subject on account of their usefulness in the determination of age of fishes. For this reason, many investigators have taken great interest in it and expressed their own opinions. The present study has shown that in section the ridge appears between two osteoblasts and it continues to grow while the inner osteoblast is losing its cytoplasm. So, there is no daubt that some of the osteoblasts which normally produce the bony layer of scale serve in making the ridges to grow. However, the above observation seems to be not sufficient basis for discussion of what induces ridge formation. The problem will be fully discussed later together with other experimental results.

As for the structure and function of the grooves, a detailed description was issued by Neave (1940). He observed in the section to which Mallory's connective tissue stain was applied, that the corresponding parts of the fibrillary plate under the grooves receive anilin blue dye in wedge form whereas the remaining part takes orange G. According to him, hardness of the scale is brought about by the infiltration of ichthylepidin, an albuminoid like collagen, into the fibrillary plate, and the peculiarity in staining at the part just under the grooves is an indication of no deposit of the substance. In considering the fact in connection with the absence of bone at those parts, he considered it as a simple form of ginglymoid arthrosis. As was described in the preceding section, also

Taylor (1916) and Wallin (1957) hold to the idea of the importance of the grooves for the flexibility of the scales. Paget (1920), Pevsner (1926) and Dietrich (1953) on the other hand thought much of the function of the grooves as a canal which serves in transporting cells probably because they found cellular elements in the grooves. The present author would conclude that the function of the grooves is to give flexibility to the scales in the light of the results described in the preceding section—the number of grooves increases along the body axis anteroposteriorly and also according to the scale growth—and in view of their structure observed in section. The cellular elements which were often observed in the grooves may probably participate in the formation of the grooves themselves. The matter will be further considered with respect to the absorption of the scale.

V. REGENERATION OF SCALES

A study of regenerated scales is convenient for elucidating the way of formation of the scale structure or in considering the essential nature of that structure owing to the characteristic features and rapid reappearance of such scales. The regeneration of scales seems to be affected by environmental factors such as water temperature or food supply (Saito and Yamada, 1953; Saito, 1955). Suzuki (1952) reported in the goldfish the complete recovery of the regenerated scales to normal in external appearance within only six months, contrary to the observation by Wunder (1949) that regenerated scales do not return to the original structure even in four years. In this section, there will be presented, in addition to a description of the process of regeneration of scales, some experimental results and a discussion concerning mainly the way of ridge formation, the effect of water temperature and feeding on regeneration, and recovery of the superficial structure of the regenerated scales.

Process of regeneration

Four ontogenetic scales adjacent to each other were removed from the upper first scale row of a goldfish, 6.1 cm in body length, and the corresponding regenerated scales were found to be at different stages of regeneration as shown in Fig. 12. The four regenerated scales (A-D) represent respectively the stages of the 4th, 5th, 6th and 7th week after the removal of the ontogenetic scales. During the period (May-June) the water temperature varied in the range from 10°C to 20°C. The regenerated scales were recognizable in the body surface before the 4th week but they were not hard enough to withstand taking out.

At the 4th week, the regenerated scale (A) was extremely thin and flexible, its longitudinal and transverse diameters showed about 80% of those of the ontogenetic (a) and there were found 3 linear ridges on it. The scale grew to about 90% of original size by the 5th week (b, B) and the number of ridges increased to 6. The regenerated scale (C) on which there were 10 ridges attained nearly the same size as compared with the normal scale (c) by the 6th week. By the 7th week, the regenerated scale (D) which was provided with 13 ridges came finally slightly to exceed the ordinary scale (d) in size. The

regenerated scales are characterized by three things; the wide central area which lacks concentric ridges but shows a grooved network, the ridges set at broad intervals in the outer of the central area, and numerous radial grooves connected with the central network. The ridges are first discontinuous and irregularly waved; the intervals between ridges become smaller and smaller as they are formed outwards arranging themselves as normal concentric ridges.

This is an example of the course of the scale regeneration which has been carried out under the above described conditions. In short, the origins of the regenerated scales were established within 3 weeks after the removal of the ontogenetic scales, thereafter, they grew rapidly during about 4 weeks until they completely recovered their original size. It was also found that the ridges were formed at the extraordinary rate of one ridge every two days during the rapidly growing stage.

The regeneration of a scale takes place essentially in the same manner as the development of an ontogenetic scale as viewed from the histogenetic standpoint. But in the case of regeneration, the formation of a new scale is carried out in almost the whole region of the emptied scale pocket (Fig. 13). A partial destruction of the epithelium which occurred with the removal of the ordinary scale is repaired within only one or two days. Active cells proliferated from the pocket wall gather in the central area of the pocket to form a widely spread plain mass. The new scale, exactly, the bony layer of it, appears in the midst of the cell mass —the developmental process is likewise carried out. However, the bony layer of the regenerated scale is not a continuous layer but is formed as an assemblage of many small platelets. So, the section of the regenerated scale in germ stage shows a horizontal arrangement of discontinuous platelets (Fig. 14). The boundaries between these platelets become later a grooved network in the central area of the scale after the addition of fibrous lamella at the lower surface. The subsequent growth of the newly formed scale which is still a thin and flexible bony structure devoid of fibrillary plate follows the ordinary course as has been observed in the case of the development of the original scales.

In the regenerated scale in surface view, the cellular elements are observed to be more abundant than in the ontogenetic one because of the rapid growth of the former (Fig. 15). Inside of each ridge particularly of the region just within the scale margin there is a row of cells adhering closely to the ridges, while the widely spread osteoblasts occupy the spaces between outer ridges. Between these osteoblasts and the remaining cells at the inside of the ridges a transformation phase can be observed. This furnishes evidence that some of the osteoblasts are transformed to the ridge formative cells as was suggested in the previous section.

Effect of water temperature and feeding on the regeneration of scales

Procedure

Early in winter, when the season causes the growth of fish to cease, twenty-four goldfish about half a year old ranging from 3.5 to 4.5 cm in body length were brought from a nursery. They were reared in the laboratory during the period from December to the following April. The fish were divided into four groups; each was kept in a

cylindrical glass tank containing five litres of water. The water temperature of two of the groups was controlled at 20°C to 22°C and the other two at 4°C to 7°C during the experimental period. One each of the two groups respectively was fed on earthworms and grain food; the other was kept under the starved condition. The fish under experiment therefore consisted of four groups; fed in warm water (WF), starved in warm water (WS), fed in cold water (CF) and starved in cold water (CS). At the beginning of rearing, fifteen scales just below the dorsal fin were removed in three rows including the lateral line from all fish. The regeneration of the scales in each fish was examined at intervals.

The material of this rearing experiment was also used in the observation on the absorption of scale and in the histochemical detection of alkaline phosphatase both of which will be described in the following sections.

Observations

When a scale is removed from the body surface, the corresponding part of epithelium and the marginal osteoblasts are also taken away together with the scale. In all fish in the four groups, the epithelial part recovered in one or two days. Ultra thin scales appeared within two weeks or so in the warm water groups whether fed or starved; on the other hand, no regeneration of scale took place in the cold water groups even after four months (three months in CF). After three months, the water temperature of CF-tank was gradually brought up to 20°C. Then, the fish in that tank regenerated their scales in twelve days. The facts reveal definitely the importance of water temperature in the regeneration of the scales.

As has already been stated, the regenerated scales are considerably smaller in size than the ordinary ones at first. They rapidly grew larger during about two weeks until their growth comes to be checked by the wall of the scale pocket. The regenerated scales which appeared in the warm water groups showed so far no indication of difference in structure between the fed and the starved groups, although those of the starved fish came to be absorbed together with the other ontogenetic scales within fifty days. No absorbed scale could be observed in the fed-in-warm-water group and the cold water groups in which regeneration did not occur. Therefore, the regeneration of the scales may have almost no connection, at least in the early stages, with the nutrition of the fish.

Recovery of the superficial structure of regenerated scales

Procedure

A new method was devised in an attempt to examine change in superficial structure of a growing scale. Its essence is the re-examination of a scale which was once removed from and then inserted again into the body surface. After once removed and after a certain period of rearing of the fish followed the insertion of the scale, the same scale at different growth stages is photographed twice at a definite magnification; the thus obtained two figures are compared and contrasted. It is necessary to take care of the inserted scale lest it fall off. For this reason, soaking of the removed scale in Ringer's solution while the photograph being taken should be avoided, though once tried; it caused the scale to drop off easily. Rather, it would be better to put the scale during the treatment on a clean slide glass without allowing it to get wet. Moreover, after prompt returning of the scale the fish should be kept quiet separately

in a small tank for one night. The presence of the scale should be confirmed before letting the fish free into the rearing pond.

In this experiment, the 5th scale of the upper first row was previously removed from each of nine goldfish in order to obtain the regenerated scale. Every four, six and eight weeks after operation, the newly formed scale was removed from three fish at a time, photographed and quickly returned to the original scale pocket. After about five months of rearing of the fish, these scales were removed and photographed again.

Results and consideration

The general aspect of the experiment is shown in Table 3. Fish No. 6 was lost. In Nos. 3, 5 and 8, the inserted scale had fallen off before the re-examination. Accordingly, out of the nine fish the once-examined scales could be re-examined on five. In all cases, the scales had continued to grow in proportion to the growth of their respective bearers. For example, the comparison of two figures in Nos. 2, 4, and 7 is presented respectively in Figs. 16, 17, and 18; each represents respectively the group of the 4th, 6th, and 8th weeks of the regeneration. There is in each scale a clear boundary which looks like an annulus between the inner old and outer new regions. This must be formed because of retardation of growth of the scale immediately following the operation such as removing and inserting. By means of this boundary one can distinguish easily the scale parts before and after the first examination.

The comparison of the first picture with the corresponding part of the second reveals the following four points: 1) The structure of the regenerated scale remains essentially the same even after about five months of growth. 2) The focal area which shows a grooved network undergoes a little change, viz., the central polygonal platelets come to be filled with a number of minute structures which are irregular elevations of bony material. These structures may be regarded as discontinuous fragments of ridges (Fig. 19). Some of the grooved boundaries between the platelets become indistinct in places and relatively smaller platelets tend to fuse into one. But careful examination of the real scale under microscope shows that the greater part of the network remains unchanged. 3) The radial grooves become clearer; most of them extend to the periphery across

Table 3. Growth of the fish in the period between first and re-examination of the regenerated scales. No. 6 was lost. In Nos. 3, 5, and 8, the once-examined scale was not re-examined. Bracketed number shows the days of regeneration in total.

Fish	First examination				Days from first to re-exam.	Re-examination		
	Days of regener.	Date	Body length (cm)	Body weight (g)		Date	Body length (cm)	Body weight (g)
No. 1	28	Jun. 15	8.2	19	156 (184)	Nov. 18	9.5	36
2	//	//	7.9	18	//	//	8.4	31
4	42	Jun. 22	9.5	33	149 (191)	//	10.1	42
7	56	Jul. 6	9.9	33	135 (191)	//	10.9	49
9	//	//	8.0	20	//	//	8.4	28

the boundary between old and new parts. A few grooves start newly at the boundary where a few others which start from the focus are interrupted. No groove is formed or disappears in the old part. 4) The arrangement of linear ridges is not altered in the least; the pattern of the already set ridges remains exactly as it was, and new ridges never appear in the focal area excepting some discontinuous irregular fragments as mentioned before.

It may safely be concluded from the above facts that the regenerated scale can by no means regain a superficial structure like that of the previous ontogenetic scale at least within six months of regeneration, although it is undeniable that a part of the grooved network disappears. The author would like to state, however, that the recovery could not take place at an indefinite future time beyond six months because the cellular elements which act in elaboration of the superficial structure should be considered to degenerate at an early stage of regeneration. When the scales were first examined, even after four weeks of regeneration, their essential pattern was found to have been already established. A little alteration in the focal area after that is nothing but the elaboration of the superficial structure by a deposit of more bony material. The appearance of fragmental elevations of bony material in the central platelets is evidence of the elaboration. Properly, these minute discontinuous fragments are expected to become usual linear ridges. In the regenerated scales, because the focal grooved area is formed instantly in a relatively wide space as a gathering of a number of bony platelets, none of them excepting the most external ones shows any directional growth owing to the reciprocal restriction. This is supposed to be the reason why the fragments appear in the focal area instead of linear ridges.

Fig. 20 shows the scale taken from fish No. 9 in which the scale was inserted obliquely being shifted from the original situation after the first examination. Probably owing to the fact that the scale had been strongly pushed against the pocket wall especially at the dorso-lateral margin, the figure (b) indicates a trace of absorption of the scale covering a considerably wide area at right. It may be considered that the scale, after the absorption had ceased, continued to grow according to the growth of the fish. In this scale, most of the grooves extend to the newly developed area keeping the changed situation, but the newly formed ridges stand out in sharp contrast to those of the older area in their direction of running; the new ridges do not grow parallel with the changed older ones but they keep the ordinary direction (compare with a). As a regenerated scale in general manifests much of the pattern of the previous ontogenetic scale as for the arrangement of ridges, consideration of the above observation may lead to the thought that the pattern of a certain scale is determined by the general form of its scale pocket and that the pattern determined by the scale pocket may not be altered easily.

Here, the possible influence of the operation, removal and insertion, on the natural growth of the scale must be considered for fear that the scale structure might be different if the scale were not taken out of the body. Most of the cells surrounding the regenerating scale are supposedly taken out together with the scale itself. It is quite possible to consider that they endure the treatment and

survive after being returned to the original pocket from the fact that a little alteration of the superficial structure does occur in the focal area. There is of course some sign of retardation of scale growth just after the treatment, but it would not seem to indicate a serious influence on the alteration of the pattern of the scale which was once taken out from the body.

Discussion

When a scale is removed from a scale pocket, a newly regenerated scale replaces it within a short period. The process of scale regeneration traces essentially the same course as that of the ontogenetic development in the young fish. Since it is carried out in the wide space of the scale pocket, the bony layer of the regenerated scale is formed as an aggregation of a number of polygonal platelets, not as a continuous plate. This was first pointed out by Neave (1940). The boundaries of these platelets remain to form a grooved network in the focal area. The fibrillary plate is added from the underlying pocket wall after the bony layer has been laid down.

The concentric ridges are set at first short and irregular at wide intervals but they change gradually to closely set continuous ridges according to the outward growth of the scale. This seems to show that the ridges are formed with difficulty while the scale is growing rapidly, but they come to get close to each other when the growth rate declines. However, it is interesting to point out that the time required for inducing the ridges in such a rapidly growing stage is very short; three or four ridges are formed within a week. In the central platelets the linear ridges are never formed but their rudiments lie scattered taking the form of a number of minute fragments. The irregular appearance of these fragments reminds one of intercellular spaces furnishing a suggestion on the manner of ridge formation.

Many workers recognized the cells attached closely inside of ridges and thought that they introduced the ridges. Klaatsch differentiated the upper cell layer of scale into the deeper and the superficial cells; the former supply the foundation of the bony layer while the latter elaborate the relief of the scale surface. Ussow agreed with him for the most part. Nusbaum as well as Neave (1936) considered differently that the cells attached inside of ridges may have an osteolytic property so the ridges are formed as much by a hollowing out behind as by a building up in front. Paget believed that the cells in question were descendants of the upper layer cells and spent their plasma in the formation of ridges leaving their nuclei inside of their own product. According to Setna, ridges are formed in a damming up of the scale margin being obstructed by the wall of the scale pocket. Neave (1940) later reconsidering his previous opinion, regarded the cells in question as osteocytes which were not buried in the bone owing to its thinness. He insisted that ridges are not the product of special cells because the superficial cells lie independently of the underlying just formed ridge but that the ridges are formed when the scale growth comes to be stagnant as a partial thickening of excessive bone formative substance which is ordinarily utilized by the growing scale margin. Dietrich follows Neave's later opinion

but she seems to admit the participation of special cells since her description is similar to that of Klaatsch. Wallin (1957) is of the opinion that normal ridges are formed only when the epidermis or the denser dermis in the scale pocket, not the scale pocket itself, forms a greater obstacle or pressure resistant to the spreading of the scale; on reaching a threshold value, periodically, the pressure induces the formation of a ridge which temporarily removes the pressure. The variety of opinion suggests the difficulty in solution of the problem.

At this point, a description on the arrangement of ridges of typical ontogenetic scale of the goldfish is required in considering the origin of ridges. In the anterior part of the scale the ridges are set close to each other parallel to the margin, while they run obliquely in the upper and lower lateral parts at a little angle to the margin. Several marginal ridges thus come to be interrupted, in turn, by the intersecting lateral margin. On this account, the ridges in the lateral parts are fewer in number and the intervals between them are wider than those in the anterior part. In the posterior part, the ridges are reduced in number to less than half of those in the lateral because some the lateral ridges come to be broken off at the border between the unexposed and exposed parts. Thus, both ends of each of the remaining few ridges meet with each other in V-shape at a point on the median line of the posterior field. There, of course, the intervals between ridges are the largest in inverse proportion to the smallest number.

Setna's view is unreasonable because the ridges of the regenerated scale are formed before the scale margin comes in contact with the pocket wall and further because they do not always run parallel with the margin. There is no doubt but that some of the osteoblasts, even if they are not specially differentiated cells as Neave insists, take part in the ridge formation. The problem is, however, in what way such an arrangement of cells is brought about.

It has already been stated that in section the ridge seems to appear between two osteoblasts so as to fill the intercellular space with bony material. As a matter of fact, however, the ridges in surface view are independent of boundaries among upper layer cells when they have just arisen at the outermost margin of the scale, that is to say, the marginal osteoblasts are not regularly arranged along the ridge but they lie across it (Neave, 1940; Wallin 1957). Therefore, it can scarcely be said that the arrangement of upper osteoblasts is responsible for the induction of ridges. Rather, it should be considered that some of the cells remain in an arrangement at the inside of ridges only to make them grow. Therefore, the essential factor to cause ridges to form should be attributed to another cause.

As was previously described, only irregular fragments are laid down in the central platelets of the regenerated scales instead of linear ridges. This suggests the absence of any factor to induce the origin of linear ridges on these platelets which cannot grow outward because of the presence of marginal platelets. Accordingly, the growth of the bone in a certain direction is supposed to induce the continuous linear arrangement of ridges on the surface of the growing scale. If the direction of running of the ridges indicates the direction of scale growth, the ridges must be set in almost perfect concentric rings because the scale is

believed to grow equally in every direction even though the anterior growth rate is slightly larger than the posterior. The ridges, however, do not really form concentric rings but run at a slightly outward angle to the margin in the lateral parts of the scale. Moreover, the arrangement of ridges in a certain scale reappears even defective in the pattern of the regenerated scale which was formed in the same position (Saito and Yamada, 1953). In the example of fish No. 9 there has been shown the inalterbility of the scale pattern of the regenerated scale which is supposed to be determined by the general form of the scale pocket.

Judging from the facts thus observed, Wallin's view seems to be very interesting. He says on the arrangement of cells, "an obstacle in the direction of the growth of the scale (dermis, inactive preparatory stage of the scale forming cells, or another factor) causes in the assemblage of cells a pressure between the obstacle in question and the calcified scale on the inside." The present author would like to consider that the "pressure" causes a part of yet uncalcified soft bone to form a fold which develops into a complete ridge with the help of inner attached cells, hereupon, the cells are considered to be caught by the fold. The scale grows larger pushing against the surrounding tissues of the scale pocket at the anterior as well as the lateral margin and against the epithelium at the posterior. As the base of the pocket wall connects with the thick fibrous corium at the anterior, there the largest pressure may be formed. The pressure may gradually decrease along the lateral margin of the scale. At the apex of the scale, where it comes in contact with the epithelium through some marginal osteoblasts, the least pressure may be formed. The variance of the pressure at different parts thus results in scales with closely paralleled ridges at the anterior part, obliquely running ridges with comparatively wider intervals at the lateral and extremely few ridges at the posterior.

The re-examination of the same scale showed that the regenerated scale never recovers the superficial structure characteristic of the ontogenetic scale. This would be theoretically reasonable because it is difficult to consider that the ridges could grow centripetally. The superficial structure of the regenerated scale is incapable of being altered after it has been established by the upper osteoblasts which disappear sooner. Therefore, the author agrees with Wunder and he cannot help doubting the statement of Suzuki who insists the complete recovery of the superficial structure of the regenerated scales within only six months. In addition to Suzuki's observation, Kobayashi and Kawai (1960) reported in their study of the regeneration of scales in the mud-loach that the central area which is devoid of ridges and grooves grows smaller with the lapse of time. Since the relative width of the central area is naturally considered to become smaller with the growth of the regenerated scale, it seems necessary to compare the width of the area with the total width of the ordinary scale not with that of the regenerated scale. According to Okabe's observation made in our laboratory (unpublished), the ratio of the grooved area of the regenerated goldfish scale, taken monthly during six months, to the total area of the ordinary scale was about 0.6~0.7 in longitudinal diameter and 0.4~0.5 in transverse diameter; there was observed no lowering of the ratio throughout the 6 months period.

Saito and Yamada (1953) reported on the scales of the crucian carp that the

regeneration takes place faster in summer than it does in winter. Further, Saito (1955) proved no influence by rearing with different foods upon the superficial structure of the regenerated scales. The rearing experiment in the present study showed that water temperature is the principal factor in inducing regenerated scales; an active metabolism in a high water temperature accelerates the regeneration of the lost scales, even if the condition forces the fish to absorb other normal scales. This suggests the necessity of active metabolism of the fish not only for the regeneration but also for the growth of the scales. However, the normal scales cannot grow without the actual growth of the fish body.

VI. ABSORPTION OF SCALES

It is well known that the absorption of scales occurs in nature as a result of severe exhaustion of fish due to an intense activity such as spawning. Absorption of scales of the goldfish can be induced when the fish is reared under malnutritive conditions or when some artificial treatment is used. In the rearing experiment described in the foregoing section some phenomena related to the absorption of scales in both the regenerated and the ontogenetic could be observed. The absorbed scales were also obtained experimentally by means of the modified re-examination method. The process of the absorption and its cause will be discussed in comparison with the formation of the scale structure in general.

Procedure

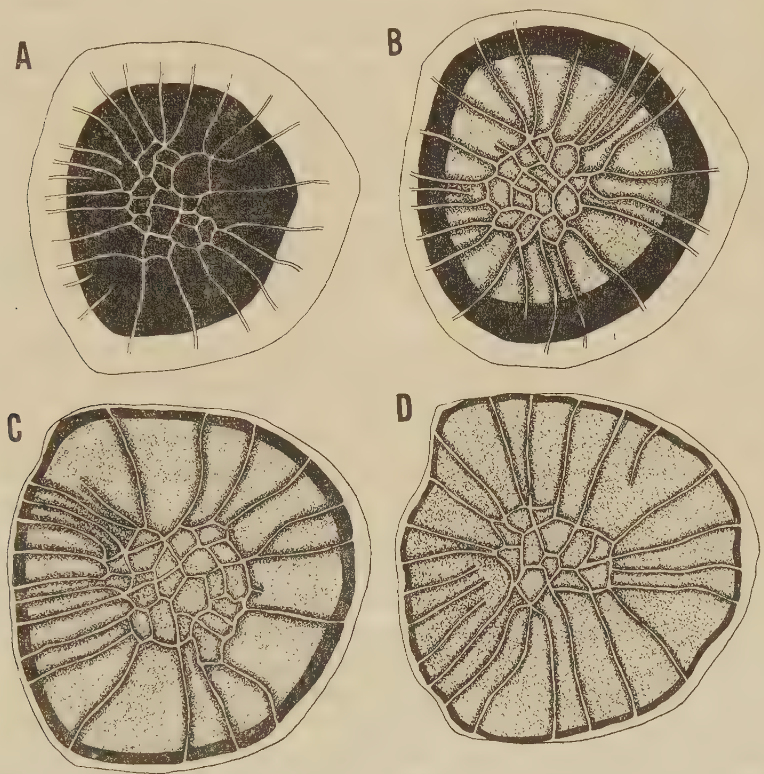
The scales under absorption were obtained from some of the fish during the rearing experiment described above in the previous section (p. 199). The scales were stained by Stoeltzner's method for the detection of the deposit of calcium salts: A fresh scale is rinsed in 2% solution of cobalt chloride for 5 minutes, so the deposit of calcium salts is changed to cobalt salts. After thorough washing in distilled water, the scale is transferred into a dilute solution of yellow ammonium sulphide, about 2 minutes; thus the site of calcium salts can be demonstrated as black precipitate of cobalt sulphide.

The essential point of the re-examination method has already been presented (p. 200), but the procedure was slightly modified in this study. 1) One scale each was removed from the middle body-part of three fish; at the anterior part of each scale a V-shaped piece was cut off. After the operation the scale which has lost most of the anterior part was returned to the original scale pocket. 2) Scales taken from another three fish were rinsed in 70% alcohol for just a short while. After washing in water they were returned promptly to the original places as usual. The scales were re-examined in the usual way after a certain period of rearing of the fish.

Results

A typical ontogenetic scale removed at the outset of the rearing and treated by Stoeltzner's method shows a deposit of calcium salts covering almost the whole surface excepting the outermost margin. The uncalcified margin in which one or two ridges are usually counted is somewhat narrower at the anterior part than at the posterior. Just inside of this margin, an intensely stained band borders

the central calcified area, and along each side of each groove are areas stained also in dark black although the groove itself is never stained (Fig. 21). This must be evidence that in the normally growing scale the just calcified margin as well as the areas along the grooves has more deposit of calcium salts than other calcified area while the outermost margin is not yet calcified.



Text-fig. 8. Diagrammatic drawings showing the order of decalcification in four stages of regenerated scale
A: 26 days of regeneration in WF
B: 50 days of regeneration in WF
C: 78 days of regeneration in WF
D: 50 days of regeneration in WS

In the warm water groups, the regenerated scales appear in a few days; they grow larger rapidly to such an extent that they attain nearly an equal size and a similar shape with the original ontogenetic scale. If these scales are stained by Stoeltzner's method, they show a relatively narrower central area stained uniformly in dark black and a wider stainless margin in comparison with the same parts of the ontogenetic scales. Such a peculiar staining of the freshly regenerated scales gradually changes to that of the ordinary scales. Take the scale at the 50th day of regeneration in the WF group for instance, the calcified zone spreads up nearly to the periphery and the colour tone of the central area

has become pale except the margin of the grooves. After 78 days, the width of the marginal dark stained band as well as that of the outermost stainless zone becomes so thin that the greater part of the scale is covered uniformly by a pale stained area (Text-fig. 8, A-C). The above observation seems to show the disappearance of the once deposited calcium salts in the central area. The process develops faster in the WS group than in the WF group. For example, in the regenerated scale taken from a fish in the WS group of 50 days rearing, there can be found not only almost no dark stained band at the periphery but also a sign of absorption occurring at the shoulders (Text-fig. 8, D). On the other hand, in the fish of the CF or CS, no regenerated scale appeared, the ontogenetic scales were never absorbed and their mode in staining showed as much the preservation of calcium salts in marginal and radial areas as did the scales taken at the outset of rearing. From the results obtained here, it may be concluded that a part of deposited calcium salts is removed prior to the actual absorption of the scale and that the process is accelerated in warm circumstances probably because of the active metabolism of the fish.

Normally, the fibrillary plate is formed at the inside of the outermost margin which consists of only the bony layer. But the corresponding section of the absorbed edge represents the inverse relation (Fig. 22). Moreover, a crack parallel with the absorbed margin is often observed on the surface of the exposed fibrillary plate which has been left behind free as a result of the absorption of the upper bony layer. In some cases the absorption of that layer takes place also on the central focal area (Fig. 23).

In the foregoing account, the absorption of the scale may be considered to consist of three processes in a broad sense, viz., the dissolution of calcium salts, the erosion of decalcified bone and the mechanical destruction of the fibrillary plate.

It was frequently observed in the regenerated scales that a certain ridge is not cut by a groove but continues across it (Fig. 24). This is quite curious because the ridges are linear elevations of bony layer while the grooves manifest a part devoid of bone. The grooves, aside from the absorbed portion, never reach up to the outermost margin, fading out in the uncalcified zone. It is quite likely, therefore, that the grooves extend as the result of the absorption of once formed bone matrix. The regenerated scales grow so rapidly until they become restricted by the scale pocket that the above phenomenon might be caused by the elongation of the groove unproportionate to the accelerated laying down of bony material. As may be seen in the figure, a part of the osteoblasts enclosing the scale margin enter into the groove. If these cells do take part in elongation of the groove, they should certainly have an osteolytic property.

Thus, the two phenomena, the absorption of the scale and the elongation of the grooves, may be considered on a common basis, that is, the erosion of bone. In this respect, the osteoclastic absorption of bone which is known as occurring in general osseous tissues comes to one's mind; the possibility of the idea will be dicussed later.

The scale from which the anterior part was cut off in V-shape shows a pair of sharp projected shoulders. The scale planted into the original scale pocket

after having been submitted such an operation was strongly absorbed particularly at the shoulders. This occurred equally in the three scales treated in that way from three individual fish. An example is shown in Table 4 and Fig. 25. The lost part of the scale is characteristic of a partial regeneration and not of an extension from the edge of the remaining part. The trace of the absorption may be seen at the scale margin extending from the shoulders to both the lateral sides and also in some of the central area. Along the ventro-lateral margin (left) is a new growth zone which shows slight growth after the absorption ceased.

Table 4. Growth of a fish used in the experiment for the examination of the fate of a scale in which the anterior part was cut off in V-shape

	Operation	Examination	Days in rearing
Date	Aug. 30	Nov. 10	72
Body length	9.9 cm	11.0 cm	
Body weight	38 g	53 g	

Table 5. Growth of a fish used in the experiment for the examination of the fate of a scale in which the cellular elements were killed with alcohol

	Operation	Examination	Days in rearing
Date	Jul. 30	Sept. 19	78
Body length	7.5 cm	8.2 cm	
Body weight	15 g	22 g	

In another experiment with three other fish, in which the cellular elements were killed by treating the scales with alcohol before insertion, the scales showed without exception a strong absorption at both the lateral parts. The result shown in Table 5 and Fig. 26 is an example from the three cases. At the anterior margin of this scale the extent of loss due to absorption is rather smaller as compared with the lateral margin, and a new growth zone develops discontinuously with the absorbed margin only at the anterior. No growth zone can be seen at any other part.

With another three fish, one of the scales was planted without receiving such operations as control for the above two experiments. Since the three scales continued to grow without showing any sign of absorption in this case (Fig. 27), it is obvious that the treatments, cutting off a part of scale or rinsing a scale in alcohol, caused the artificial absorption. It should be noticed that unlike the ordinary absorption which is caused by some environmental factors, other scales of the same individual fish are still growing larger while the scale in question is being absorbed. In the former experiment, the pocket wall which was stimulated by the sharpened tips of the shoulders would probably give rise to the absorption of the scale. It has been seen in Fig. 20 that such a stimulation caused the absorption of the regenerated scale at the part where a strong pressure may have been formed. It is a matter of course that the part lost in the V-shaped cutting was repaired by a partial regeneration because edge of the remaining part has no peripheral osteoblast. The junction between the regener-

ated and the remaining parts was also absorbed considerably. This would be caused in the necessity of joining the old part to the newly formed part. In the latter experiment, on the other hand, the scale was planted without hurt but the marginal osteoblasts were killed with alcohol. As the scale was washed before planting, it is impossible to suppose any effect of alcohol in stimulating the pocket wall. The dead scale having no power to grow by itself is nothing but an obstacle to the scale pocket, so the strongly absorbed scale may be an expression of the effort of the pocket which worked to eliminate the obstacle.

Discussion

Ichikawa (1953) reported similar results to the above on the disappearance of calcium salts prior to the destruction of the scales of the carp under starvation. On the surface of those scales he observed a remarkably thinner calcified layer compared with that of normal scale and considered that a mechanical breaking would occur on the softened scale due to the absorption of calcium salts from the fibrillary plate. The dissolution of a part of deposited calcium salts is taking place even under normal condition, but it is accelerated in warm circumstance with insufficient nutrition probably because of the active metabolism of the fish. Evidence for this statement is shown in the fact that the actively calcifying zone gives a stronger reaction being stained darker than the central calcified zone. Wallin (1957) proposed in his study of the roach that the width of the osseoid zone (the outermost uncalcified zone) or of the zone of calcification (dark stained zone) tells whether the fish is actually in the growing state or not, because he observed the fact that both the zones increase in width when the scales are growing rapidly. The present observation on the transition in staining reaction of the regenerated scales confirms his proposition. It was also proved that scleroprotein, the ground substance of bone, is produced by the active osteoblasts at the scale margin while calcium salts secondarily deposit on it at just inside of the margin. According to another report of Ichikawa (1956), a part of the deposit of calcium salts in goldfish scales is considered to be replaced gradually by new elements since he found a deposit of injected radioactive strontium in the inner part of the scales. Therefore, it seems probable that there is always a repetition in depositing and dissolving calcium salts over the whole surface of the scales and that a turnover of calcium ions is always taking place between the scales and the surrounding tissue fluids.

The idea of mechanical breaking proposed by Ichikawa may be applied only to the fibrillary plate but not to bone. The term "mechanical" seems to imply a rubbing action of the shrinking scale pocket, but the experimental results showed that the absorption of scale can be induced in the growing fish. Osteoclasts, usually multinucleated giant cells having an osteolytic function, are known in general osseous tissues especially where the absorption is taking place. The nature of their function or the stimuli which cause them to form still remain as unsolved questions. They are regarded as arising from the fusion of relatively immobile connective tissue cells or of mobile cells such as macrophage or even from any cells, and such transformation of cells is said to be reversible (Hancox, 1949). The formation of osteoclasts in scale pockets from osteoblasts or from

fibroblasts under some stimulation appears therefore to be quite likely. It was found in this study that the stimulation which caused the scale to be absorbed was a strong pressure against the scale pocket in one case or the killing of the cellular elements of the scale in another case. The absorption brought about by starvation is similar to the former case.

The absorption of bony layer occurs also in the focal area. According to Yasuda (1958), scales of the crucian carp reared under a mal-nutritive condition for three years showed in the central area a network like that of the regenerated scales. As he did not illustrate the scales the present author cannot offer any comment about that, but he supposes that the partial absorption which occurred in the focal area of those scales probably presented such a network, so they should be different from the regenerated scales in structure because the former ones are caused in the partial destruction of bone while the latter ones consist of complete bone which only lacks linear ridges.

VII. SOME BIOCHEMICAL ASPECTS OF THE SCALE GROWTH

Alkaline phosphatase and glycogen

The protein constituent of teleost scales is reported to be composed 76% of collagen and 24% of ichthylepidin; the latter is also an albuminoid intermediate between collagen and keratin (Green and Tower, 1901). In general in the osteogenic process, alkaline phosphatase is known to play an important role in producing protein and the subsequent ossification. The occurrence of a considerable amount of glycogen prior to the calcification has also been recognized in many osseous tissues. Since it was interesting to ascertain whether or no alkaline phosphatase and glycogen exist in the scale forming cells, as in other osseous tissues, histochemical tests for those substances were applied to tissue sections of goldfish skin.

Procedure

The materials used in this study were obtained also from the fish which were being reared under the various conditions to investigate the regeneration of scales (p. 199). Small pieces of skin taken from these fish were fixed and embedded in paraffin. As fixatives, Bouin's and Helly's fluids were used for the histological preparations, cold 80% alcohol or acetone for the histochemical detection of alkaline phosphatase, and Gendre's modification of Allen-Bouin's fluid for that of glycogen respectively. In the histochemical test for alkaline phosphatase, use was made of Gomori's calcium-cobalt technique, sodium-β-glycerophosphate buffered pH at 9.0 as the substrate. For the demonstration of glycogen, Bauer's cromic acid-Schiff reaction and Best's ammonia carmine reagent were used on the collodionized tissue sections. The usual histological stainings such as Ehrlich's or Delafield's haematoxylin, Mallory's triple connective tissue stain, and Heidenhain's azan stain were also employed. Besides these, the measurement of hydrolytic power of alkaline phosphatase in the kidneys of the fish under the experimental rearing conditions was performed *in vitro* to find out any possible relationship between the enzymic activities of the kidneys and the environmental factors.

Observations

As has been stated already, the rearing was carried on dividing the fish into four groups; two groups in warm water (20°C) and two groups in cold water (5°C) respectively with or without food supply. Probably because of the smallness of the tanks neither the growth of the bodies nor that of the scales could be observed in the groups during the experimental period.

The histochemical test for alkaline phosphatase applied to the skin of these fish proved that the enzyme exists only in the epithelial cells but not in the cells or tissues surrounding the scales (Fig. 28). There was observed no difference in staining reaction with the fish under different rearing conditions. It has already been mentioned that no regeneration of scale occurred in the cold groups even after four months of rearing contrary to the rapid appearance of the regenerated scales in the warm water groups (see p. 200).

On certain days after the removal of scale, the corresponding skin pieces in which no regenerated scale was observed were examined in the same way. After 26 days, for example, the operated area is covered completely with regenerated epithelial cells including a neuromast under which is an accumulation of multiplied fibroblast; the enzyme is detected only in the epithelial cells but not in the fibroblasts (Fig. 29). Similarly, after 78 days, the scale pockets are filled with cells, but in them no alkaline phosphatase can be proved (Fig. 30). These fish regenerated the lost scales when the water temperature was raised to 20°C. Both the fibroblasts in the wall of the scale pocket and the osteoblasts which adhered to the newly formed scales showed a clearly positive reaction of the enzyme (Fig. 31).

Therefore, there is no doubt but that the occurrence of alkaline phosphatase in the scale-forming cells is a necessary factor to induce the regeneration of scales and probably to make them grow. The absence of the enzyme in the cells associated with the ontogenetic scales being in restrained growth is very suggestive of its importance in producing the proteins which constitute the ground substance of the scales.

Table 6. Alkaline phosphatase activity of the kidneys of the fish reared for 50 days under various conditions. Eighteen hours incubation at 36°C in a substrate containing sodium-β-glycerophosphate in veronal buffer adjusted pH at 9.0. Colorimetric measurement was performed by Youngburg & Youngburg's method. WF: warm water (20°C) with food supply; WS: warm water without food supply; CF: cold water (5°C) with food supply; CS: cold water without food supply

Condition of rearing	Figh		Org.-P in substrate	Inorg.-P liberated per 10 mg of kidney, less than control	Org.-P hydrolysed
	Body length (cm)	Body weight (g)	(mg)	(mg)	(%)
WF	4.45	2.50	0.054	0.036	66.1
WS	4.00	1.65	〃	0.032	58.8
CF	4.00	2.80	〃	0.019	34.9
CS	4.50	3.60	〃	0.005	9.2

As the appearance of regenerated scales was assumed to be influenced by water temperature, it was questioned whether any correlation exists between the phosphatase activity and the environmental conditions. In this account, the kidney was chosen for a study of the activity of the enzyme because it was supposed to possess rather abundant alkaline phosphatase and to be sensitive to the metabolic activity of the fish. Measurements were made on the kidneys obtained from fish at 50 days of rearing in each group. The water-extracted enzyme was added to a substrate containing a known amount of phosphoric ester, and after incubation for 18 hours at 36°C liberated inorganic phosphate was measured by means of a photoelectrocolorimeter. The result is shown in Table 6. The fatness of the fish in the warm groups is conspicuously lower than that of those in the cold; the enzymic activity of the kidney decreases in the order of WF, WS, CF and CS. This is evidence showing an intense metabolic activity of the fish in comparatively higher water temperature. Also feeding seems to have considerable, though less, influence on the metabolism of the fish. The difficulty of scale regeneration in the fish at low temperature is supposed to be caused by the weakness to synthesize intracellular alkaline phosphatase due to the inactivation of internal metabolism.

As to the histochemical detection of glycogen, both the methods yielded negative results even in the cells associated with the regenerating scales in which the presence of alkaline phosphatase was proved.

Polysaccharides and ribonucleic acid

The occurrence of mucopolysaccharides in the form of chondroitin sulphate has been known in various hardened tissues in connection with the calcification (Logan, 1935; Partridge, 1948; Dziewiatkowsky; 1951; Bradbury, 1958). Besides the presence of alkaline phosphatase, it is reported that a high content of ribonucleic acid (RNA) is generally found in protein-forming cells and that there is a direct correlation between the content of RNA of the cells and their capacity for synthesizing proteins (Brachet, 1950). Histochemical examinations for these substances were thus attempted to the normal and the regenerated scales.

Procedure

Pieces of the goldfish skin containing either normal or regenerated scales were fixed in Allen-Bouin's and Carnoy's fluids or in lead acetate-formalin mixture after Lison-Sylvén (Glick, 1949). Sections prepared by paraffin method were treated with McManus' periodic acid-Schiff (PAS) procedure to demonstrate polysaccharides. The sections were also stained with toluidine blue to render metachromasia visible. Staining with methyl green-pyronine after Unna-Pappenheim was employed for the identification of RNA.

Results

Both the bony layer and fibrillary plate show a strong reaction to the PAS regardless of the state of calcification. Fig. 14 shows a section of a regenerated scale to which the PAS was applied. The reaction is negative at the part of the fibrillary plate just under the grooves. The difference in intensity of the reaction cannot be identified as due to the two different layers. The sections of a normal scale stained with toluidine blue show neither orthochromasia nor metachromasia

excepting only the lowest (newest) fibrous lamella which shows orthochromasia. When the sections of the scale which had been decalcified with HCl were treated with toluidine blue, the bony layer manifested metachromatic staining. Similarly, the regenerated scale in germ stage which is not yet calcified also shows metachromasia. This proves in the bony layer the presence of acid mucopolysaccharide bounded by calcium ions.

The active osteoblasts around the rapidly growing regenerated scales show feeble pyroninophilia (Fig. 32); the staining reaction disappears when the section is treated with 5% trichloroacetic acid in order to remove RNA from it. On the contrary, the osteoblasts enclosing the periphery of the normal ontogenetic scales do not show such an affinity with pyronine. These findings indicate that the contents of RNA in the scale-forming cells is intimately connected with their ability to synthesize proteins.

Parathyroid hormone

Parathyroid hormone is known to control the concentration of blood calcium in higher vertebrates. Parathyroidectomy results in lowering the serum calcium level (Collip, 1925). Hypertrophy of the glands affects skeletal tissues by causing removal of calcium salts from them and the implantation or the tissue culture of the gland in contact with bone induces the absorption of the bone (Barnicot, 1948; Gaillard, 1955). The presence of parathyroid glands in fish is not so far confirmed. Only Schereschewsky* (1940) found in *Lebistes* a glandular epithelium, at the isthmus between first gill arches, which he insisted was the parathyroid. For this reason, an injection experiment of the hormone prepared from horses was performed in an attempt to ascertain the possible effect of the hormone on the fish scales.

Procedure

Parathyroid hormone was prepared according to Tweedy's procedure (Tweedy, 1930) from the glands taken from horses.** From 40 g of the glands 16.5 mg of gray amorphous powder was obtained. Ringer's solution containing 0.3 mg/ml was injected everyday for 42 days into three fish so as to correspond to 0.1 mg per 100 g of the body weight at a time, and their scales were examined weekly by applying Stoeltzner's method.

Result

The fish as well as their scales continued to grow normally during the experimental period as compared with the control. The absorption of their scales could not be observed. The regeneration of the scales which were removed for weekly examination took place as usual, and no difference in the manner of calcification was observed in comparison with those of the control fish. After all, no effect on the scales of the repeated injection could be observed.

Discussion

The presence of alkaline phosphatase, acid polysaccharide and ribonucleic

* According to Kawamoto (1956).

** The collection of the glands and the extraction of the active substance were performed through the aid of Mr. T. Fukazawa of the Faculty of Agriculture, Hokkaido University, Mr. H. Mori of the Ebetsu Health Center, and Mr. S. Nakatsubo of the Sansho Pharmaceutical Co. Ltd., to whom the writer expresses his heartfelt thanks.

acid was proved in the goldfish scales and the associated cells. This suggests that the scales, at least their bony layer, is formed by a biochemical mechanism much the same as that of other osseous tissues, although no glycogen was proved and no effect of parathyroid hormone could be observed.

The mechanism of the hardening of osseous tissues is not so far quite solved. Robison and his collaborators (Robison, 1923; Robison and Soames, 1924; Martland and Robison, 1924; Fell and Robison, 1933; 1934) offered an explanation of it from the evidence that increase in amount of alkaline phosphatase and glycogen is recognizable in the hypertrophic cartilage cells. They believed that alkaline phosphatase is concerned in the process of ossification by exerting effect on phosphoric esters in the blood, thus bringing about a local increase in the concentrations of inorganic phosphate ions, so that the product of the concentrations of phosphate and calcium ions becomes greater than the solubility product of calcium phosphate which is thereupon deposited in the solid state. It was supposed that hexosemonophosphoric esters liberated in the phosphorolytic process of glycogen may be the substrate for phosphatase (Harris, 1932). Nowadays, however, since it is known that bone salt is not a simple form of calcium phosphate but that it consists of submicroscopic crystals of hydroxyapatite $[Ca_3(PO_4)_2]_3 \cdot Ca(OH)_2$ (Hendricks and Hill*, 1950), the process of the deposition of calcium salt is considered to be not so simple as described above. At any rate, there is no doubt but that alkaline phosphatase plays an important role in the deposition of the calcium salt by liberating phosphate ions. There are a few reports concerning alkaline phosphatase related to fish scales (Roche *et al*, 1940; Wallin, 1957). Roche *et al* found that the activity of the enzyme in *Clupea pilchardus* varied according to seasons, that is, the activity in the period from May to July rises up to ten or twenty times that in winter. Wallin failed to demonstrate the enzyme histochemically in the scale-forming cells of the roach and concluded that alkaline phosphatase has no bearing on the formation of the scales. He considered that Roche *et al* had measured an activity of the enzyme in the epithelium because he found strong activity thereof in the epithelium. The invariable activity of alkaline phosphatase in the epithelial cells of the goldfish was proved also in the present study. It was shown distinctly that the osteoblasts around the regenerated scale which is in an actively growing stage contain a considerable amount of the enzyme. Further, the occurrence of alkaline phosphatase in the original papilla cells was recognized in the rainbow trout, in spite of the absence of this enzyme in the epithelium of this species (Yamada, 1956). Wallin's failure is probably due to the fact that he applied the reaction to cells which were in a state either inactive or active but not sufficiently sensitive to react. This interpretation may be applied to the explanation of the negative reaction of alkaline phosphatase as well as RNA in the normal ontogenetic scales. Løvtrup (1959) found in a study of amphibian embryogenesis that the activity of alkaline phosphatase was retarded or blocked at low temperatures when the morphological development was delayed or stopped. The difficulty of scale regeneration at low temperature is thus a result of a low chemical activity which is reflected in the

* Cited from Maximov and Bloom (1952).

facts that the enzyme was histochemically absent in the associated tissues and that the kidney showed a low enzymic activity *in vitro* in case of the fish kept at a low temperature.

The absence of glycogen in the scale-forming osteoblasts at any stage seems strange because glycogen is generally found as the source of the substrate for alkaline phosphatase in every osseous tissue which shows the occurrence of the enzyme. Wallin also could not detect glycogen. The fact is unexplainable so far the methods for glycogen were correctly employed. Only it may be possible to consider that phosphate ester already decomposed is carried by humours into the areas where the scales are growing.

The positive reaction of PAS in both the bony layer and the fibrillary plate suggests the presence of mucopolysaccharides as a cementing substance among collagen fibrils. As the reaction is not influenced by the state of calcification this type of polysaccharides may probably have no connection with the calcification. The negative reaction at the part of the fibrillary plate just under the grooves is interesting in considering the possible function of the grooves. A metachromatic staining in addition to the PAS on the decalcified bony layer and on the regenerated scales implies the presence of acid polysaccharides, especially of chondroitin sulfate, as it was reported that metachromasia is weakened *in vitro* when calcium ions are added to chondroitin sulfate (Levine and Schubert, 1952). The fibrillary plate does not show metachromasia; this suggests some other calcifying mechanism in this structure differing from that in the bony layer. Wallin is of the same opinion as he found many large crystals, the so-called Mandlian bodies, in the fibrillary plate. Kato (1953) proved that the Mandlian bodies are crystals of calcium oxalate which were thought to be rare in the animal kingdom. This together with the fact that it lacks chondroitin sulfate, makes the fibrillary plate to be conspicuous among calcified tissues. The orthochromasia of the newest fibrous lamella shows its basophilic nature which is soon lost probably on account of what was considered by Neave as an infiltration of ichthylepidin.

A high concentration of alkaline phosphatase and RNA in the osteoblasts around the regenerated scales is evidence of active protein synthesis, that is, the formation of collagen fibrils, occurring in these cells. It is still uncertain in what way these substances participate in the synthesis of proteins. It may be said without doubt that the occurrence of these substances in the scale-forming cells is a necessary condition for the growth of the scales. Concerning the formation of collagen fibrils in fibrogenic cells, for reference, Jackson (1954) reported in a bone rudiment of fowl embryo that the cytoplasm of the osteoblasts is packed with long submicroscopic filaments which may become extracellular fibrils of the bone matrix. It was also found by him (1955) that there are present in fibrogenic cells some cytoplasmic granules which contain polysaccharide protein components and give alkaline phosphatase reaction but no specific staining for nucleic acids. He assumes the function of the granules that they may secrete the ground substance of bone, they may synthesize the collagen fibrils, or they may contain precursors for the complex extracellular material.

Wallin made an injection experiment on the roach using AT 10, an anti-

tetanic drug which acts similarly to the parathyroid; he found that it caused a conspicuous rise in the content of serum calcium within 48 hours after injection. He concluded in his account that there is no essential difference between the fish and higher animals in reacting to a preparation of the character of the parathyroid hormone. Since he did not make any comment about the effect of the substance on the scales and since in the present study the measurement of serum calcium was not made, it is impossible to compare the two results with each other. However, as Wallin made research on this problem in his study of fish scales, the present author supposes that he could not observe any positive effect of AT 10 on the scales. In the present case, on the other hand, it is uncertain whether such a rise of serum calcium level might be caused to occur or not. After all, the growth of the scales was by no means affected by the injection of the hormone prepared from the glands of horses.

VIII. GROWTH OF SCALES ANALYZED BY MEANS OF TRANSPLANTATION

The transplantation of scales in the goldfish has been undertaken with success by several workers. Mori (1931) observed the transformation of the ordinary scales when they were transplanted into the lateral line. Goodrich and Nichols (1933) described the effect on chromatophores and the tissue reactions following after auto- or homoioplastic transplantation of scales among various types of goldfish. Besides these works on the transplantation of an individual scale, there are other studies concerning the transplantation of a patch of skin including several scales (Sauter, 1934; Nardi, 1935). But there is no report which analyzed the appearance of the superficial pattern of scales by means of transplantation. In this section, the observation on the growth of transplanted scales which was traced according to the re-examination method will be presented.

Procedure

A general account of the experiment is shown in Table 7. A certain scale in each of nine fish was removed from its scale pocket and another smaller scale taken from the same individual (autoplastic transplantation) in Nos. 1-6, or from another smaller individual (homoioplastic transplantation) in Nos. 7-9, was transplanted into that scale pocket. The ordinary and donated scales were photographed, the latter one before planting. The transplanted scales were chosen so as to be rather smaller in Nos. 1-3, moderately smaller in Nos. 4-6 and remarkably smaller in Nos. 7-9 than the ordinary scales. After a certain period of rearing of the fish, the transplanted scales were removed and photographed again. Then the obtained three figures from one fish, that of the ordinary scale, the transplanted scale before planting and the same scale after rearing, were compared with each other. A tracing paper on which was copied the outline of a figure was employed in the comparison putting the paters one upon another figures.

Results

The fish may be divided into three groups, in each of which are three fish,

Table 7. Growth of the fish used in the experiment of scale transplantation, and the comparison in size of the ordinary and the transplanted scales. Autoplastic transplantation in Nos. 1–6. Homoioplastic transplantation in Nos. 7–9, the transplanted scales were taken from a fish, 4.3 cm in body length.

Fish	Rearing period	Days in rearing	Before rearing		After rearing		Diameters of ordinary scale		Diameters of transpl. scale		l/L	t/T
			B.L.	B.W.	B.L.	B.W.	Long. (L)	Trans. (T)	Long. (l)	Trans. (t)		
			cm	g	cm	g	mm	mm	mm	mm		
1	May 14 Nov. 18	188	9.8	36	10.8	54	5.9	6.8	5.8	5.3	0.98	0.79
2	May 14 Nov. 18	188	9.8	35	10.5	48	6.1	5.9	5.8	5.0	0.95	0.85
3	May 14 Sept. 19	128	8.1	18	9.1	30	4.7	4.6	4.7	4.2	1.00	0.92
4	Aug. 29 Nov. 10	73	9.6	36	10.3	48	6.0	6.0	5.0	4.9	0.83	0.81
5	May 15 Nov. 18	187	8.2	19	9.0	26	4.5	4.6	3.4	3.6	0.76	0.77
6	Sept. 1 Nov. 10	71	8.8	31	9.2	33	5.4	6.5	4.3	3.6	0.79	0.75
7	Jul. 3 Nov. 18	138	7.3	14	8.3	26	4.2	4.4	2.0	2.5	0.48	0.57
8	Jul. 3 Nov. 18	138	6.9	11	7.9	21	3.4	4.1	2.0	2.4	0.58	0.59
9	Jul. 3 Nov. 18	138	6.2	8	8.1	21	2.8	3.9	2.0	2.4	0.70	0.62

according to the ratio of the two diameters of an ordinary scale to that of the transplanted scale. The results will be described in turn for each group.

No. 1 - No. 3 (Figs. 33-35): In each fish, the ordinary scale was the 6th of the upper first scale row while the transplanted scale was the one from the 20th of the same row, so the latter is nearly the same as the former in longitudinal diamter but is slightly smaller in transverse. Therefore, the transplanted scale showed a larger growth after rearing of the fish at both the lateral margins than at the anterior where the width of the extension is variable in detail, viz., the donated scale has a tendency to approach the ordinary scale in form adapting itself to the general form of the scale pocket of the host. Table 8 shows the

Table 8. Width of growth and number of ridges formed after the transplantations at the anterior and the lateral parts of the transplanted scales in Nos. 1–3. W: width of growth; R: number of ridges

Fish	Anterior growth				Lateral growth			
	Max.		Min.		Upper		Lower	
	W. (mm)	R.	W. (mm)	R.	W. (mm)	R.	W. (mm)	R.
No. 1	0.36	17	0.15	8	0.53	13	0.49	12
2	0.19	7	0.11	5	0.37	11	0.43	12
3	0.28	11	0.07	2	0.20	3	0.50	12

width of growth at different parts and the number of ridges formed in the corresponding parts. It is obvious from the table that the scales showed a better growth at the margin where the scale pockets supposedly offered them more space to grow. The ridges were formed almost in proportion to the width of growth. The distance between ridges is wider at the lateral margin than at the anterior, but this is common in usual goldfish scales and is not limited particularly to the transplanted ones. Rather, it should be emphasized that the space in the scale pocket exerted almost no effect on the spacing of ridges though it allowed a better growth of the scale at the lateral part. The examination by making use of tracing paper proved that the transplanted scales in No. 2 and No. 3 barely attained approximately the same size and form as the ordinary scale after 6 or 4 months, despite the fact that the transplanted scales should grow larger than the ordinary scales because the fish showed a considerable increase either in body length or in body weight during the period (Table 7). Accordingly, it can hardly be said that the transplanted scales grew faster than other scales of the same fish even though such scales are not restricted by the wall of the scale pocket. The position of the grooves in the donated scales was not altered; they extend straight outward keeping their ordinary situation. This seems to indicate that the scale pocket has no definite pattern as to the induction of the grooves and that the osteogenic cells may easily be transformed into osteolytic cells.

No. 4 - No. 6 (Figs. 36-38): The ordinary scale removed was the 6th of the upper first scale row while the donated scale was taken from the same position of the upper 4th row; the latter corresponded to about 70~80% of the former in longitudinal or transverse diameter. As the experiment failed in No. 4 and No. 6, two other fish were recruited in the course of rearing. In No. 5, which was reared for a longer period than the other two, the transplanted scale showed a marked growth around the margin particularly at the ventro-lateral probably because the scale was planted in a pocket biased dorsalward (Fig. 37). Intervals between ridges in the ventro-lateral field where was found the largest growth, were distinctly wider than those in the inner part of that field and also in the corresponding part of the ordinary scale, while in the anterior and dorso-lateral fields, the ridges lie in uniform spacing being unaltered. The innermost one of the widely set ridges in the ventro-lateral field is somewhat disturbed. It reminds one of an incomplete ridge which is generally seen in the inner region of regenerated scales. Just inside of the ridge, there can be seen a trace of absorption which occurred along the old margin of the transplanted scale. In short, a considerable gap in the scale pocket around the transplanted scale induced a larger growth, and the accelerated growth affected the arrangement of ridges only at the ventro-lateral field where the largest growth was obtained accompanied with a partial absorption of the inner part of the field. With the other two recruited fish, the rearing period was short and the transplanted scales showed little growth. The result obtained from the scale of No. 4 seems to be quite similar to that from No. 2 or No. 3, viz., the scale gets close to the ordinary scale in outline but being still smaller than the ordinary scale and the arrangement of the newly formed ridges is not affected by the larger scale pocket (Fig. 36). Fish No. 6 showed almost no growth of the body owing to the

injurious effect of a wooden rearing tank to such an extent that this and another fish were kept whilst the latter died early. The transplanted scale also showed practically no growth (Fig. 38). Both in No. 4 and No. 6, absorption of the old margin could not be found. Therefore, it may be said that the transplanted scales in these fish grew in proportion to the growth of the bodies without showing any partial absorption along the old margin.

No. 7 - No. 9 (Figs. 39-41): The 6th scale of the upper first row was removed from three fish and into the scale pocket respectively, was transplanted a scale taken from nearly the same position of another fish, 4.3 cm in body length. The donated scales showed equivalent to 50~60% of the ordinary scales in longitudinal and transverse diameters. In all cases, the transplanted scales presented the same results. The new growth zone of these scales showed obviously a superficial pattern characteristic of regenerated scales. So, the scales take an appearance of a regenerated scale on which the transplanted scale was placed, but no polygonal platelets can be seen in the central region and the number of grooves is not so great as compared with a typical regenerated scale. The examination of these scales under microscope with various focusing proved that the two zones, inner transplanted zone and outer newly formed zone, are in the same horizontal level, suggesting that the scale pocket into which was inserted the smaller scale did not induce another new scale, that is, these curious scales would be formed in cooperation of the transplanted scale with the scale-forming tissue of the host. The margin as well as the surface of the transplanted zone presents a sign of severe absorption. The scales may be regarded, therefore, as an exaggeration of the one obtained in fish No. 5. Each scale has surpassed in growth the size of the ordinary scale.

Discussion

It may be said generally, in summarising the observations obtained with No. 1 - No. 9, that if the scale pocket is larger in comparison with the transplanted scale a larger growth is induced. However, the transplanted scales do not always grow rapidly until they come to be restricted by the pocket wall. Examples of such a situation may be seen in Nos. 2, 3, 4 and 6. In these scales, the ridges were formed closely with each other their ordinary arrangement being unaltered. In Nos. 5, 7, 8 and 9, on the other hand, the growth zone of the transplanted scales showed widely set ridges. They all grew beyond the size of the ordinary scales in spite of the fact that they were remarkably smaller than the ordinary scales when they were transplanted. This shows unusually rapid growth of these transplanted scales. It must be noticed here that there always occurred a partial absorption of the transplanted scale in connection with the rapid growth. According to Goodrich and Nichols, who studied the homoiotransplantation of scales among several varieties of the goldfish, the absorption of the transplanted scales represents the degree of incorporation between the scales and their hosts. The absorption which occurred in the above mentioned scales of the present experiments may be interpreted in this light. When the transplanted scale is not so small in size compared with the ordinary scale, the scale pocket reacts in receiving the new scale as if it were an ordinary one, and the scale grows larger keeping in

step with other scales of the same fish. So, it is reasonable that the transplanted scale in No. 6 showed almost no growth because practically the fish did not grow. In such a case, the ridges are formed at usual intervals being not influenced by the wider pocket. On the contrary, when the transplanted scale is considerably smaller and is incompatible with the scale pocket which properly has ability to form an entirely new scale, it comes to be partially absorbed. It appears, therefore, that the absorption of the non-compatible scale is required for the reconstruction or reorganization of the scale-forming cells in the pocket from which the ordinary scale has been lost, and only in this reorganized cell mass around the transplanted scale can its rapid growth be introduced. The ridges are hardly formed in such a rapidly growing zone, but they come gradually to be formed in contiguity when the growth slackens.

Here arises a question, viz., the rapid growth accompanied by the absorption of the transplanted scale in Nos. 7-9 might be caused by homoiotransplantation and not by the smallness of the scale, because in Nos. 1-6 the scale was taken from the same individual whereas in Nos. 7-9 from another. Goodrich and Nichols reported on homoiotransplants in various combinations among different types of goldfish, that the dissolution of scale occurred in 9 out of 85 scales when the transplants were made from the type gold to gold in contrast to none in the case of autotransplants. Therefore, the question seems not to be groundless. At any rate, it does not matter so far the absorption is cosidered as a representation of incompatibility between the transplanted scale and the scale pocket.

The author interpreted in a foregoing section the arrangement of ridges, borrowing from the opinion expressed by Wallin; viz., it seems to be brought about by a pressure which is formed between a growing scale and the surrounding tissues and that pressure decreases from the anterior to the apex. This interpretation would be correct as to the arc-shaped bending of ridges and it should be further supplemented concerning the spacing of ridges in the light of the results obtained in the transplantation experiments; that is to say, the distance between ridges or the number of ridges formed in a definite width of the scale is determined exclusively by the growth rate of the scale itself. Rapid extension of the scale-forming cells or tissues prior to the actual growth of the scale is supposed to reduce the pressure formed between the tissues and the scale. In this respect, Neave's opinion—the ridges are formed in a partial accumulation of excessive bony substance which is normally utilized in extending the scale margin—may be acceptable. As the rapid growth of scales is necessarily brought about by the rapid growth of fish body, excepting the case of regeneration of scales, the distance between ridges may be generally regarded as an indication of the growth rate of fish. In this connection, the feeding experiment on the rainbow trout reported by Gray and Setna (1930) is very suggestive. They obtained scales with abnormally wide intervals between ridges even during winter by supplying the fish with abundant food and scales with abnormally narrow rings by supplying scanty diet. They suggested in view of such results that the width of ridges is closely associated with the growth rate of fish.

On account of the facts presented here, it is incorrect to consider the growth of scale separately from the growth of the fish since the scale is not a wast product

of metabolism such as a nail or hair a view frequently held.

IX. GENERAL CONCLUSION

The structure and growth of the goldfish scales have been examined from various aspects and discussions based on each examination have been presented separately. So, it will be needed here to offer a collective consideration on the formation or growth mechanism of each of the constituents of the scale, and grounded upon such consideration the usefulness of the scale for age determination should be determined.

The goldfish scale just like other teleost scales is composed of two different layers, the upper bony layer and the lower fibrillary plate. The bony layer bears on its surface a number of concentric ridges which are linear elevations homogeneous with the bony layer, while the fibrillary plate is built up of several thin lamellae. The former increases in width by its marginal growth in almost uniform thickness, but the latter grows both in width and in thickness by the addition of successively larger new lamellae. Calcium salts deposit on the bony layer and the intimate lamellae of the fibrillary plate. The development or extension of the bony layer, which corresponds morphologically as well as physiologically with that of a membrane bone in general, occurs always in precedence of and is necessarily accompanied with the addition of new fibrous lamellae. The scale originates from an aggregation of osteoblasts which have been derived from fibroblasts which migrated from the lateral line region. The osteoblasts which are divided into two layers by their own product, the origin of the bony layer, come to behave in different ways; the upper layer cells degenerate sooner with some of them remaining inside of the ridges whereas the lower ones remain longer forming a fibrous lamella. This situation is kept at the scale margin as long as the scale grows larger. On the other hand, at the lower central region of the scale is formed a new fibrous lamella in expense of underlying fibroblasts which do not pass through the stage of osteoblasts. The cellular sheath surrounding the growing scale is thus composed of marginal osteoblasts and a central fibrous lamella, and it takes part in making the scale grow while another new sheath is prepared. It is natural, therefore, that a close relation is found between the growth of the bony layer and that of the fibrillary plate, so the scale may be regarded as having a distinctive structure in this respect among skeletal tissues. The peculiality of the fibrillary plate is also found in the fact that it is lacking in acid polysaccharide although calcium salts are deposited on it.

The growth of the scale is in proportion to the growth of the fish body; regional differences in the extension of the body surface lead to the variable forms of the scales which have adapted themselves to their respective scale pockets. The width of growth in the anterior part of the scale is slightly larger than in the posterior judged from the transition in position of the focus.

The origin of the ridges is considered to be a fold of bony substance formed as a result of an obstacle of surrounding cells or tissues of the scale, not by the restrictive action of the scale pocket upon the growing scale. The fold grows into a complete ridge in increasing its height in consumption of the cytoplasm of a row of osteoblasts caught by the fold. The fold hardly occurs when the scale is

growing rapidly, and the rapid growth of the scale, except in the case of the regeneration, becomes possible only when the body grows rapidly. On the contrary, retarded growth of the scale which follows retarded growth of the fish induces on its surface the formation of closely set ridges. After all, it would be safe to say that the growth rate of the fish exerts effect upon the spacing of ridges which are being formed on its scales.

It can generally be said that the grooves in the scale are increased in number with the growth of the scale by the appearance of incomplete grooves. They extend outward probably in dissolving once-formed bony material at the scale margin. Their function is supposed to be giving flexibility to the scale judging from their histological structure and from the fact that the number of grooves of the scales covering the flexible part of the body is greater than in those of other parts. The scale pocket has no special structure to effect the pattern of arrangement of the grooves.

When a scale pocket loses its ontogenetic scale, it is immediately supplied with a new regenerated scale, and when a scale becomes obstacle for the scale pocket, it is absorbed until the obstacle is removed. The facility in producing or resorbing of the scale suggests the possible mutual conversion between osteogenic and osteolytic cells. This explanation is also applicable to the appearance or disappearance of the grooves. The regenerated scale never recovers its superficial pattern characteristic of the original ontogenetic scale. This is reasonable from the viewpoint of the mechanism of ridge formation. A high metabolism rate of the fish in a high water temperature accelerates the regeneration of the scale. One of the reasons is considered to be related to the ability to synthesize intracellular alkaline phosphatase in the scale-forming cells.

From the facts considered above, it may generally be accepted that the so-called "winter ring" or "annulus" is formed from autumn to winter with decline in the growth rate of scale, and a zone marked by wide intervals of ridges appears from spring to summer due to increase in the growth rate. But it should be emphasized that winter or summer as such does not necessarily have any effect on the scales to make the ridge intervals narrow or wide, because the spacing of ridges is in no direct correlation with the seasons but it only reflects the growth rate of the scales or of the body. A suitable water temperature with sufficient nutrition would allow the fish to grow fully, but a higher temperature with insufficient nutrition would not only arrest the growth of the fish but also would sometimes cause reduction in the weight of the fish. At that time, narrow ridge intervals like a winter ring or a disturbance of ridges formed after the resorption of the scales, both likely to be mistaken as a year ring, may occur even in summer.

In conclusion, it may be said generally that the scales are useful for the age determination of the goldfish so far as the fish shows annual periodicity in its growth rate. In this connection, a careful consideration of the ecology of the fish or of the environment should be required in order that one may avoid any errors which are thought to be probable in scale reading. This conclusion may be applied not only for the goldfish but also for other teleosts since all teleost scales are considered to be essentially the same in structure as well as in growth mechanism.

LITERATURE

Barnicot, N. A. (1948). The local action of the parathyroid and other tissues on bone in intracerebral grafts. *Jour. Anat.* 82, 233–241.

Brachet, J. (1950). *Chemical embryology.* 2nd Edit. 522p. New York; Interscience Publishers.

Bradbury, S. (1958). A cytological and histochemical study of the connective tissue fibres of the leech, *Hirudo medicinalis. Quart. Jour. Micr. Sci.* 99(2), 131–142.

Collip, J. B. (1925). The extraction of a parathyroid hormone which will prevent or control parathyroid tetany and which regulates the level of blood calcium. *Jour. Biol. Chem.* 63(2), 395–438.

*Creaser, C. W. (1926). Structure and growth of scales of fishes in relation to the interpretation of their life-history, with special reference to the sunfish *Eupomotis gibbosus Univ. Mich. Misc. Pubs.* 17.

Dietrich, M. A. (1953). A histological study of the development of the scales of the largemouth bass (*Micropterus salmoides*). *Quart. Jour. Micr. Sci.* 94(1), 71–82.

Dziewiatkowsky, D. D. (1951). Isolation of chondroitin sulfate-S from articular cartilage of rats. *Jour. Biol. Chem.* 189(1), 187–190.

Fach, M. (1937). Zur Genese der Fischschuppe. *Zeitschr. Anat. Entw. Gesch.* 107, 65–67.

Fell, H. B. & Robison, R. (1933). Glycogen and cartilage. *Nature* 131, 62.

——— & ——— (1934). The development of the calcifying mechanism in avian cartilage and osteoid tissue. *Biochem. Jour.* 28(6), 2243–2252.

Gaillard, P. J. (1955). Parathyroid gland tissue and bone *in vitro*. *Exp. Cell Res. Suppl.* 3, 154–169.

Glick, D. (1949). *Technique of histo- and cytochemistry.* 531p. New York; Interscience Publishers.

Goodrich, H. B. & Nichols, R. (1933). Scale transplantation in the goldfish *Carassius auratus*. *Biol. Bull.* 65(2), 253–265.

Gray, J. & Setna, S. B. (1930). The growth of fish. IV. The effect of food supply on the scales of *Salmo irideus*. *Jour. Exp. Biol.* 8, 55–62.

Green, E. & Tower, R. W. (1901). The organic constituents of the scales of fishes. *Bull. U.S. Fish. Commiss.* 21, 97–102.

Hancox, N. M. (1949). The osteoclast. *Biol. Rev.* 24(4), 448–471.

Harris, H. A. (1932). Glycogen in cartilage. *Nature* 130, 996–997.

Hase, A. (1911). Die morphologische Entwickelung der Ktenoidschuppe. *Anat. Anz.* 40, 337–356.

*Hendricks, S. B. & Hill, W. L. (1950). The nature of bone and phosphate rock. *Proc. Nat. Acad. Sci., Geophysics* 36, 731.

Ichikawa, R. (1956). A study of fish scale by radioactive strontium. Isotope Kenkyu-Riyo Soran, Nippon Genshiryoku Sangyo Kaigi (Congress of Japan Atomic Energy Industry). 551–552. (in Japanese).

——— (1953). Absorption of fish scale caused by starvation. *Rec. Ocean. Works Jap., N.S.* 1(2), 101–104.

Jackson, S. F. (1954). Fibrogenesis in connective tissues. *Nature* 173, 950–951.

——— (1955). Cytoplasmic granules in fibrogenic cells. *Nature* 175, 39–40.

Kato, K. (1953). Calcium oxalate and other calcium salts in fish scale. *Sci. Rep., Saitama Univ., Ser. B.* 1(2), 51–58.

Kawamoto, N. (1956). *Fish physiology.* 319p. Tokyo; Ishizaki Shoten (in Japanese).

Klaatsch, H. (1890). Zur Morphologie der Fischschuppen und zur Geschichite der Hartsubstanzgewebe. *Morph. Jahrb.* 16, 209–258.

Kobayashi, H. (1958). Comparative morphology and hierarchy of fish scales. *Bull. Aiti Gakugei Univ.* 7, 1–104. (in Japanese).

——— & Kawai, M. (1960). Studies on the regenerative scales of fishes. II. On the regenerative scales of mud-loach, *Misgurnus fossilis anguillicaudatus* (*CANTOR*). *Bull. Jap. Soc. Sci. Fish.* 26(3), 207–210. (in Japanese).

Levine, A. & Schubert, M. (1952). Metachromasy of thiazine dyes produced by chondroitin sulfate. *Jour. Amer. Chem. Soc.* 74(1), 91–97.

Lindahl, P. E. & Wallin, O. (1955). On the relationship between the growth structure of the bony and the fibrillary layers of the roach-scale. *Arkiv f. Zool., Ser. 2.* 8(8), 561–563.

Lison, L. (1953). *Histochimie et cytochimie, Principes et méthodes.* 2e Édit., 607p. Paris; Gauthier-Villars.

Logan, M. A. (1935). Composition of cartilage, bone, dentin, and enamel. *Jour. Biol. Chem.* 110(2), 375–389.

Løvtrup, S. (1959). Utilization of energy sources during amphibian embryogenesis at low temperatures. *Jour. Exp. Zool.* 140(3), 383–394.

Martland, M. & Robison, R. (1924). The possible significance of hexosephosphoric esters in ossification. V. The enzyme in the early stages of bone development. *Biochem. Jour.* 18(6), 1354–1357.

Maximov, A. A. & Bloom, W. (1952). *A textbook of histology.* 6th Edit., 616p. Philadelphia and London; W. S. Saunders Co.

Mori, Y. (1931). On the transformation of ordinary scales into lateral-line scales in the goldfish. *Jour. Fac. Sci., Imp. Univ. Tokyo, Sect. IV. Zool.* 2(3), 185–194.

Nardi, F. (1935). Das Verhalten der Schuppen erwachsener Fische bei Regenerations- und Transplantationsversuchen. *Roux Arch. Entw. Mech.* 133, 621–663.

Neave, F. (1936). The development of scales of *Salmo*. *Trans. Roy. Soc. Can., Sect. V.* 30(3), 55–72.

——— (1940). On the histology and regeneration of the teleost scale. *Quart. Jour. Micr. Sci.* 81(4), 541–568.

Nusbaum, J. (1907). Materialien zur vergleichenden Histologie der Hautdecke der Wirbeltiere. III. Zur Histogenese der Lederhaut und der Cycloid-Schuppen der Knochenfische. *Anat. Anz.* 30, 297–310.

Paget, G. W. (1920). Report on the scales of some teleostean fish with special reference to their method of growth. *Fish. Invest., Ser. 2.* 4(3), 1–28.

Partridge, S. M. (1948). The chemistry of connective tissues. 1. The state of combination of chondroitin sulphate in cartilage. *Biochem. Jour.* 43(3), 387-397.

Petrov, V. V. & Petruschewsky, G. K. (1929). Beiträge zur Kenntnis der Struktur der Schuppen von *Cyprinus carpio L.* *Zool. Anz.* 84, 257–269.

Pevsner, V. (1926). Zur Frage über die Struktur und die Entwickelung der Schuppen einiger Knochenfische. *Zool. Anz.* 68, 303–313.

Robison, R. (1923). The possible significance of hexose phosphoric esters in ossification. *Biochem. Jour.* 17(2), 286–293.

——— & Soames, K. M. (1924). Ditto. II. The phosphoric esterase of ossifying cartilage. *Ibid.* 18(3/4), 740–754.

Roche, J., Collet, J., & Mourgue, M. (1940). Activité phosphatasique et croissance des os dermique (Écailles) chez les poissons (Sélaciens et Téléostéens). *Enzymologia* 8(4), 257 260.

Saito, S. (1955). On the mechanism of the appearance of the scale structure. III. On the regeneration of the scales of the crucian carp, *Carassius carassius* (*L.*), when they are reared with the different food. *Bull. Fac. Fish., Hokkaido Univ.* 5(4), 332–335.

——— (1956). Ditto. IV. A study on the scale structure of the crucian carp, *Carassius carassius* (*L.*), *Ibid.* 7(2), 85–95.

Saito, S. & Yamada, J. (1953). Ditto. II. The scale structure of the crucian carp examined with the regeneration. *Mem. Fac. Agr., Hokkaido Univ.* 1(4), 555–560. (in Japanese).

Sauter, V. (1934). Regeneration und Transplantation bei erwachsenen Fischen. *Roux Arch. Entw. Mech.* 132, 1–41.

*Schereschewsky, H. (1940). Über den Bau des Epithelgewebes in der pharyngeal Kiemenregion der Knochenfische. *Doklady Akad. Nauk, USSR., N.S.* 29, 165–168.

*Setna, S. B. (1934). Development of the trout scale. *Jour. Univ. Bombey* (*Biol. Sci.*) 2(5), 17–32.

Suzuki, R. (1952). The change in structure of regenerated scales of goldfish. *Jap. Jour. Ichthy.* 2(4/5), 192–195. (in Japanese).

Taylor, H. F. (1916). The structure and growth of the scales of the squetague and the pigfish as indicative of life history. *Bull. Bur. Fish.* 34, 289–330.

Tweedy, W. R. (1930). Studies on the plasma calcium raising principle of bovine parathyroid glands. 1. A method of preparation and some observation on the yield, solubility and stability of the product. *Jour. Biol. Chem.* 88(3), 649–657.

*Ussow, S. A. (1897). Die Entwickelung der Cycloid-Schuppe der Teleostier. *Bull. Soc. Imp. Nat. Moscou* 1897, 147–148.

Wallin, O. (1956). Mucopolysaccharides and the calcification of the scales of the roach (*Leuciscus rutilus*). *Quart. Jour. Micr. Sci.* 97(3), 329–332.

——— (1957). On the growth structure and developmental physiology of the scale of the fishes. *Rep. Inst. Freshw. Res., Drottingholm* 38, 385–447.

Wunder, W. (1949). Beobachtungen über Schuppen-regeneration beim Goldfisch (*C. auratus L.*), beim Karpfen (*C. carpio L.*) und bei der Brachse (*Abramis brama L.*). *Roux Arch. Entw. Mech.* 143, 396–470.

Yamada, J. (1956). On the mechanism of the appearance of the scale structure. V. The possible role of alkaline phosphatase in the formation of the teleost scale. *Bull. Fac. Fish., Hokkaido Univ.* 7(3), 185–201.

——— & Saito, S. (1952). Ditto. I. The early development of the scale in the rainbow trout, *Salmo irideus G. Mem. Fac. Agr., Hokkaido Univ.* 1(3), 354–360. (in Japanese).

Yasuda, H. (1958). Implication of the scale ring. I. The phenomenon of unformed rings on the scales. *Bull. Jap. Soc. Sci. Fish.* 24(8), 613–615. (in Japanese).

(* not available)

Explanation of Plates

PLATE I

Figs. 1–3. Sections through the skin of a goldfish, 12 mm in body length.

1. The skin composed simply of the epithelium and corium. Haematoxylin and eosin. ×250 ep: epithelium; co: corium
2. Two adjacent scale papillae appearing between the epithelium and corium. Haematoxylin and eosin. ×250 sp: scale papilla
3. The scale papillae in which appeared a fine crack. Haematoxylin and eosin. ×320 cr: crack

Figs. 4–6. Sections through the skin of a goldfish, 15 mm in body length.

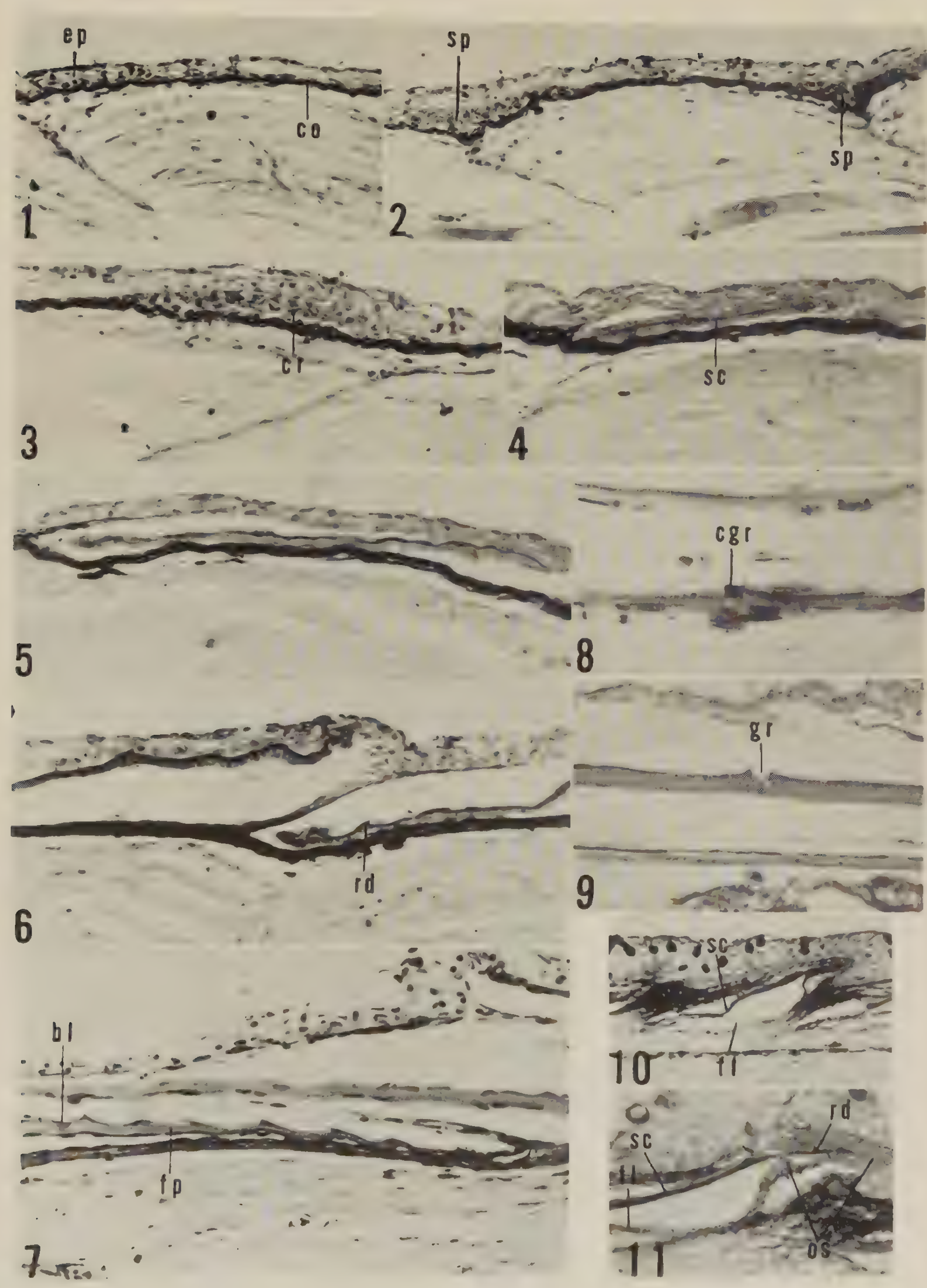
4. The origin of scale enclosed by flattened papilla cells (osteoblasts). Haematoxylin and eosin. ×320 sc: scale origin
5. A stage of the development of scale showing the degeneration of upper osteoblasts. Haematoxylin and eosin. ×320
6. Posterior end and anterior ends of two adjacent scales separated by the wall of scale pocket. Haematoxylin and eosin. ×320 rd: ridge

Figs. 7–9. Sections through the skin of a goldfish, 21 mm in body length.

7. Anterior part of the completely developed scale composed of the bony layer and fibrillary plate. Spicular projections of ridges are seen on the scale surface. Haematoxylin and eosin. ×320 bl: bony layer; fp: fibrillary plate
8. A groove in section at the peripheral part of scale. A cell square in form is visible in the groove. Haematoxylin and eosin. ×800 cgr: cell in groove
9. A groove in section at the inner part of scale. Haematoxylin and eosin. ×320 gr: groove

Figs. 10–11. Sections through the skin of the rainbow trout, 38 mm in body length, to show the manner of formation of fibrous lamella. Haematoxylin.

10. ×250 fl: fibrous lamella; sc: scale
11. ×640 fl: fibrous lamella; os: osteoblasts; rd: ridge; sc: scale



J. Yamada: Studies on the Structure and Growth of the Scales

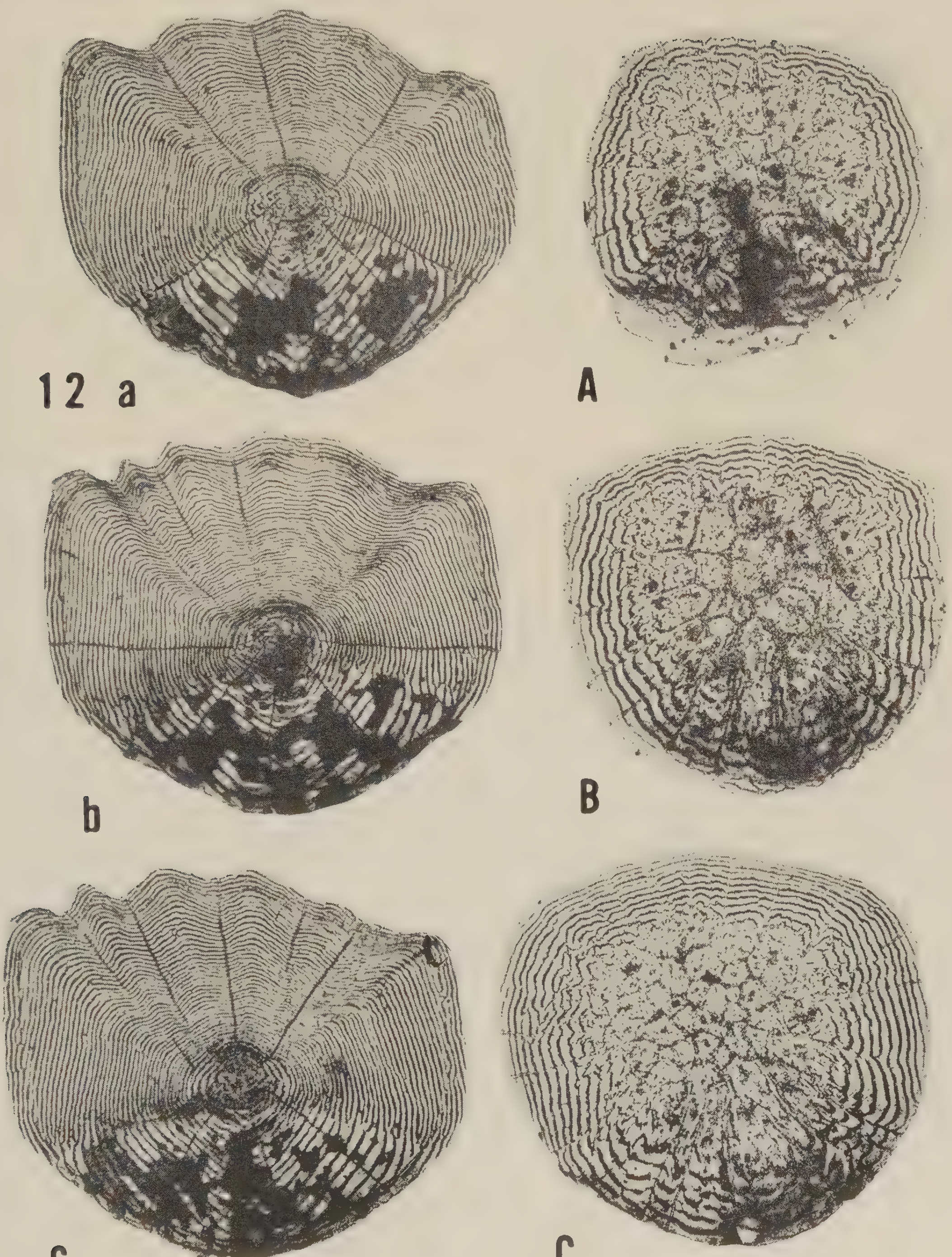
PLATE II

Fig. 12. (continued on Plate III) Four ontogenetic scales (a–d) removed from the upper first scale row of a goldfish, 61 mm in body length, and the corresponding regenerated scales (A–D) at different stages of regeneration. ×18

a, A Ontogenetic scale (a) and the regenerated scale (A) four weeks after the removal of a.

b, B Ontogenetic scale (b) and the regenerated scale (B) five weeks after the removal of b.

c, C Ontogenetic scale (c) and the regenerated scale (C) six weeks after the removal of c.



J. Yamada: Studies on the Structure and Growth of the Scales

PLATE III

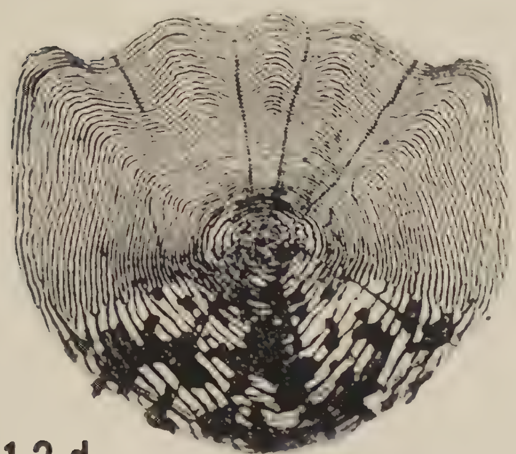
Fig. 12. (continued from Plate II) ×18

d, D Ontogenetic scale (d) and the regenerated scale (D) seven weeks after the removal of d.

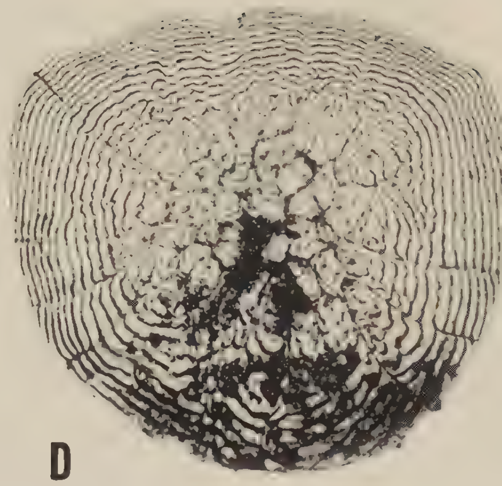
Fig. 13. A section through the regenerating scales. Haematoxylin and eosin. ×120 sc: regenerating scales

Fig. 14. A section through the focal area of a regenerating scale showing discontinuous platelets in horizontal arrangement. PAS. ×250 pl: platelets of regenerating scale

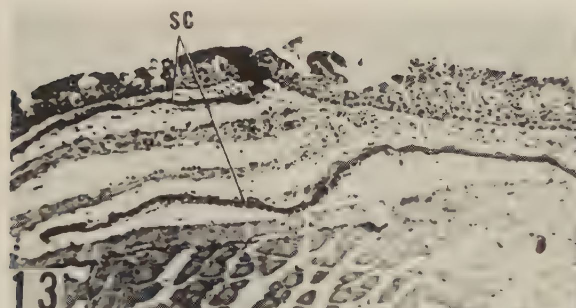
Fig. 15. Surface view of a part of the regenerated scale in flat mount. Mallory. ×400 er: erythrocytes; os: osteoblasts; rdc: cells remaining at the inside of ridges

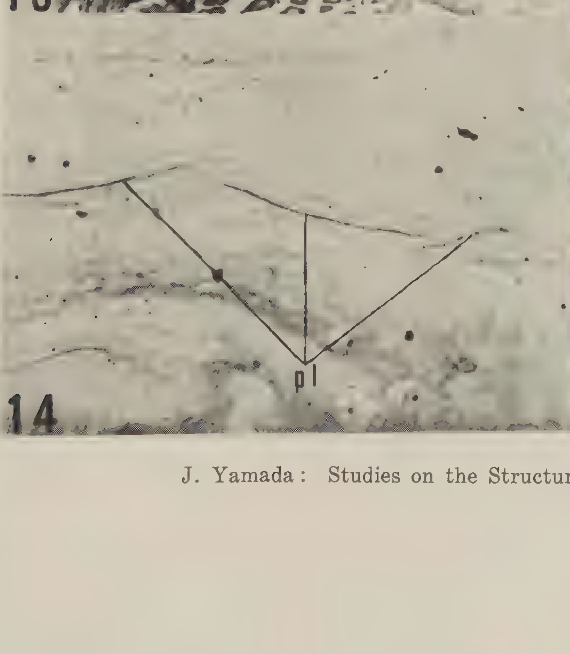


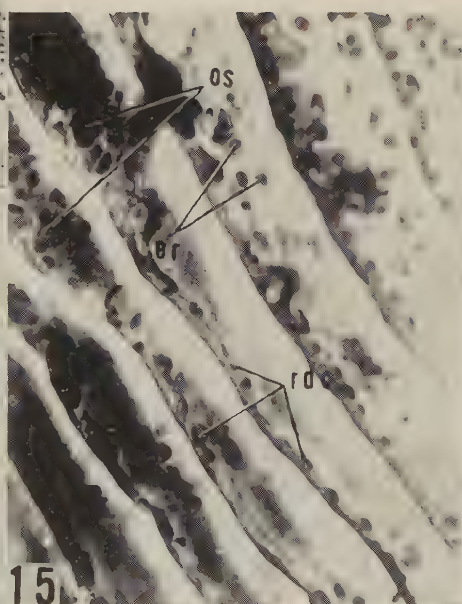
12 d



D







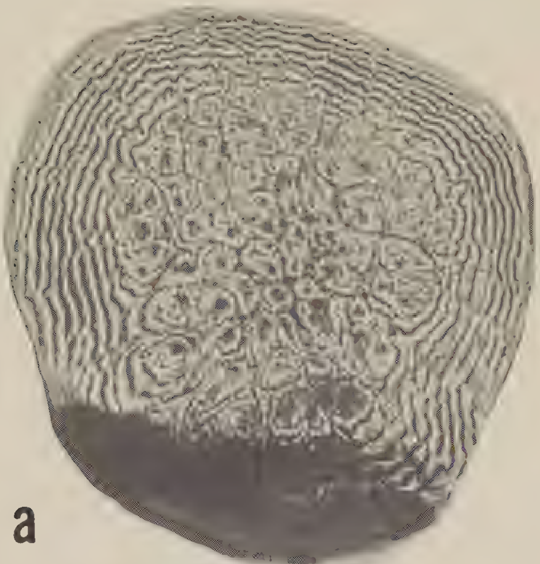
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE IV

Fig. 16. Two stages of the regenerated scale in fish No. 2, specifications shown in Table 3. ×18

a First examination at four weeks of regeneration.

b Re-examination 156 days later.



16 a



b

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE V

Fig. 17. Two stages of the regenerated scale in fish No. 4, specifications shown in Table 3. $\times 18$

a First examination at six weeks of regeneration.

b Re-examination 149 days later.



17a



b

J. Yamada: Studies on the Structure and Growth of the Scales

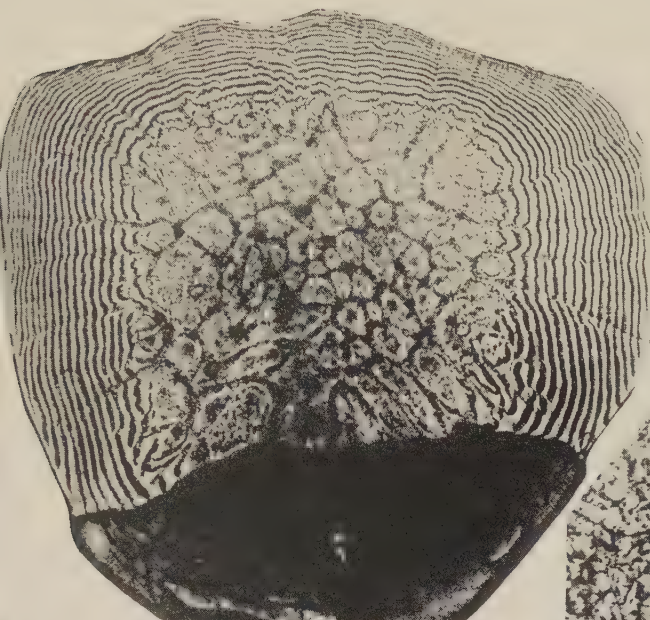
PLATE VI

Fig. 18. Two stages of the regenerated scale in fish No. 7, specifications shown in Table III. ×18

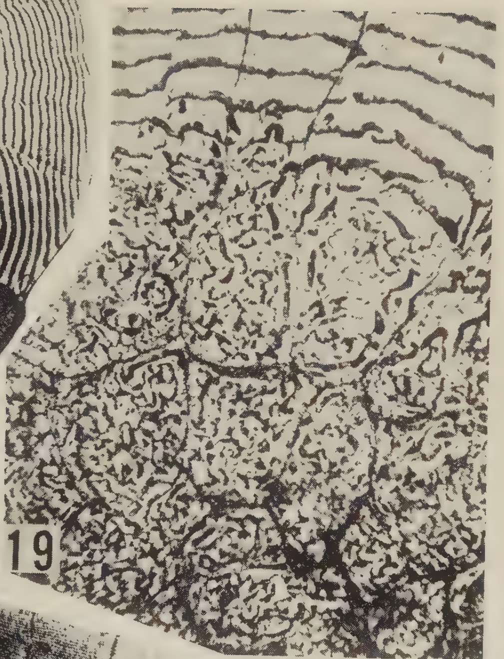
a First examination at eight weeks of regeneration.

b Re-examination 149 days later.

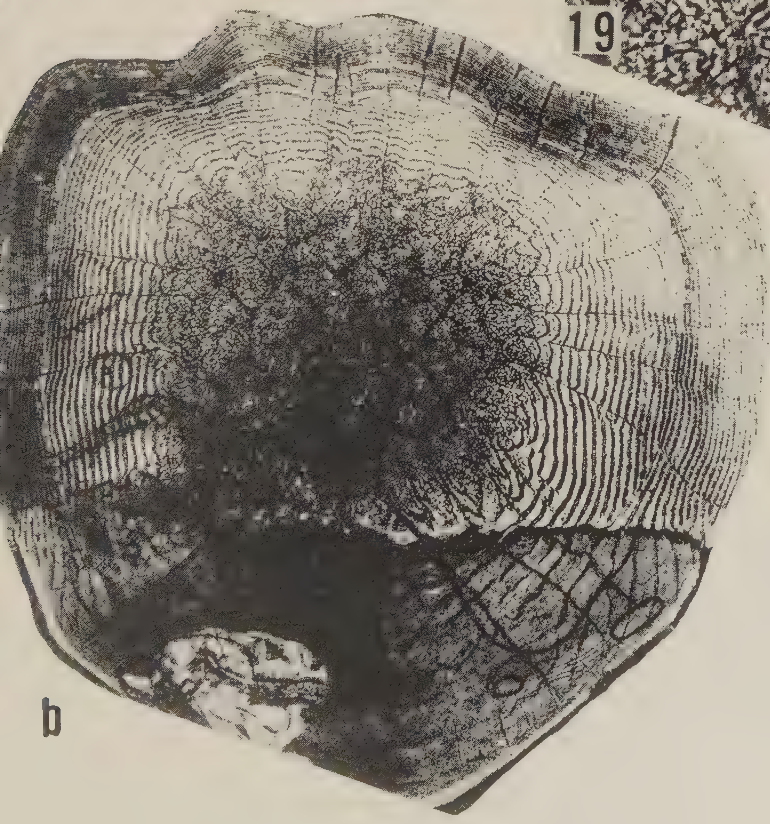
Fig. 19. Magnification of the focal area of regenerated scale to show a plenty of irregular fragments which are gradually transformed to linear circular ridges. ×250



18 a



19



b

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE VII

Fig. 20. Two stages of the regenerated scale in fish No. 9, specifications shown in Table 3. ×18

a First examination at eight weeks of regeneration.

b Re-examination 135 days later.



J. Yamada: Studies on the Structure and Growth of the Scales

PLATE VIII

Fig. 21. Typical ontogenetic scale stained with Stoeltzner's method to show the deposit of calcium salts in the marginal area and along the grooves. ×40

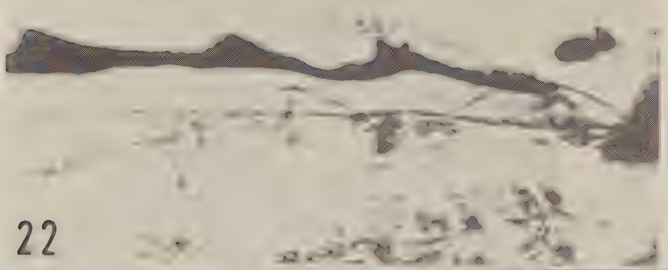
Fig. 22. Sections of an absorbed scale margin. Stoeltzner. ×600

Fig. 23. Portion of ontogenetic scale showing the absorption which occurred in the focal area. Mallory. ×60

Fig. 24. Phase-contrast photomicrograph of the margin of a regenerated scale. Mallory. ×200


21


23


22


24

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE IX

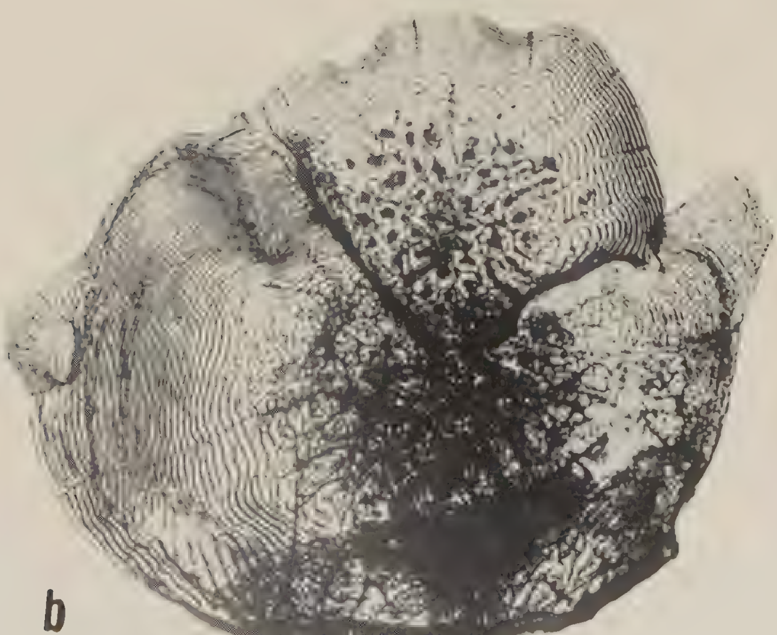
Fig. 25. Two stages of the scale in which the anterior part was cut off in V-shape. ×18

a First examination when the operation was made.

b Re-examination 72 days later.



25 a



b

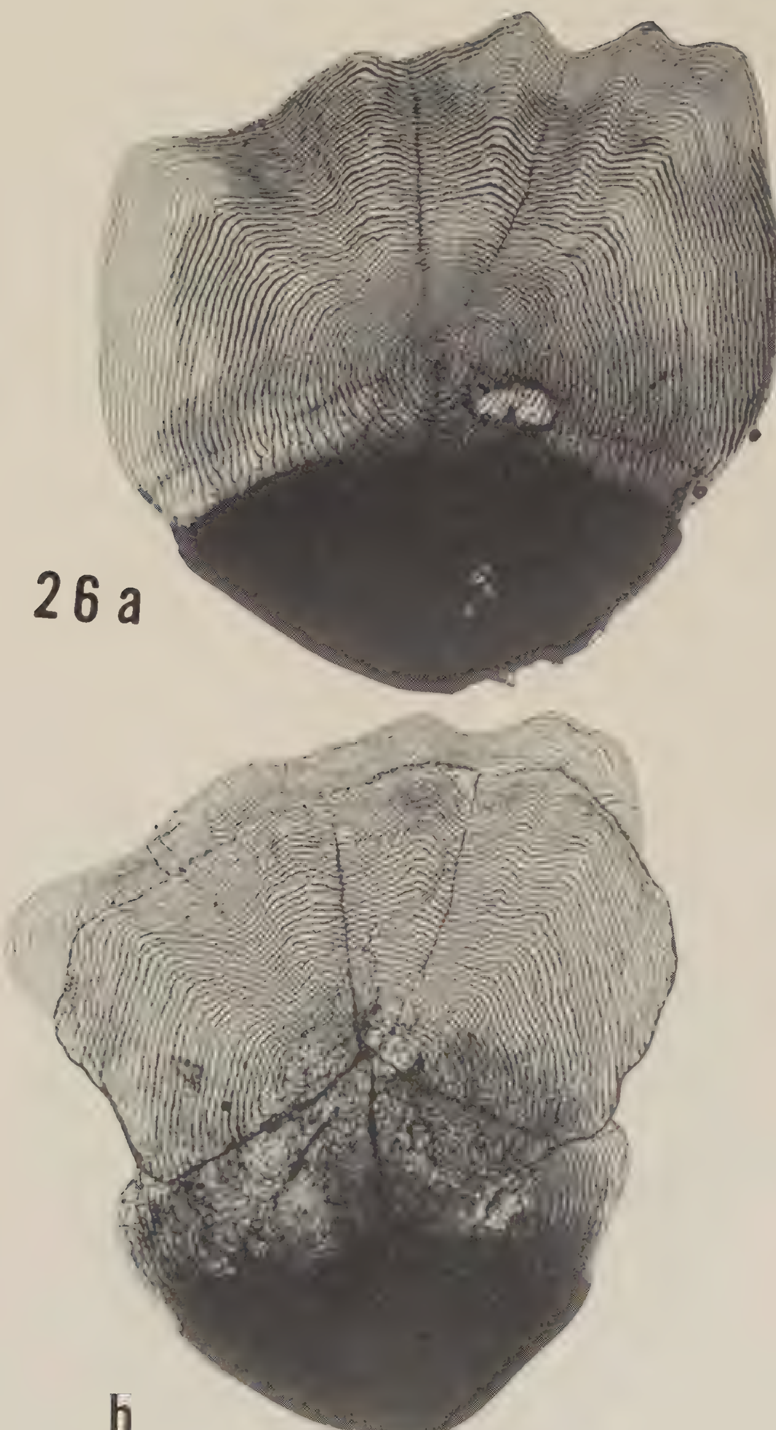
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE X

Fig. 26. Two stages of the scale in which the cellular elements were killed by rinsing in alcohol. $\times 18$

a First examination just after the treatment.

b Re-examination 78 days later.



26 a

b

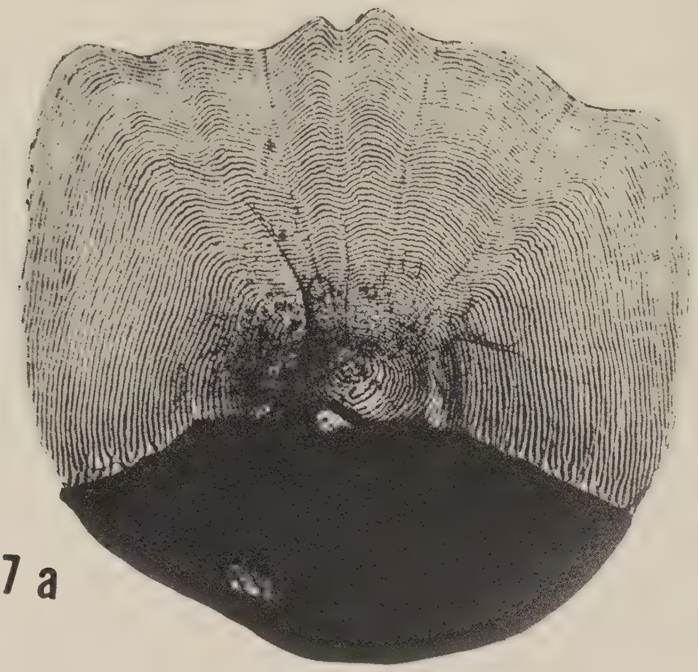
J. Yamada : Studies on the Structure and Growth of the Scales

PLATE XI

Fig. 27. Two stages of the scale removed and inserted to the original place without any treatment. ×18

a First examination.

b Re-examination 188 days later.



27 a



b

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XII

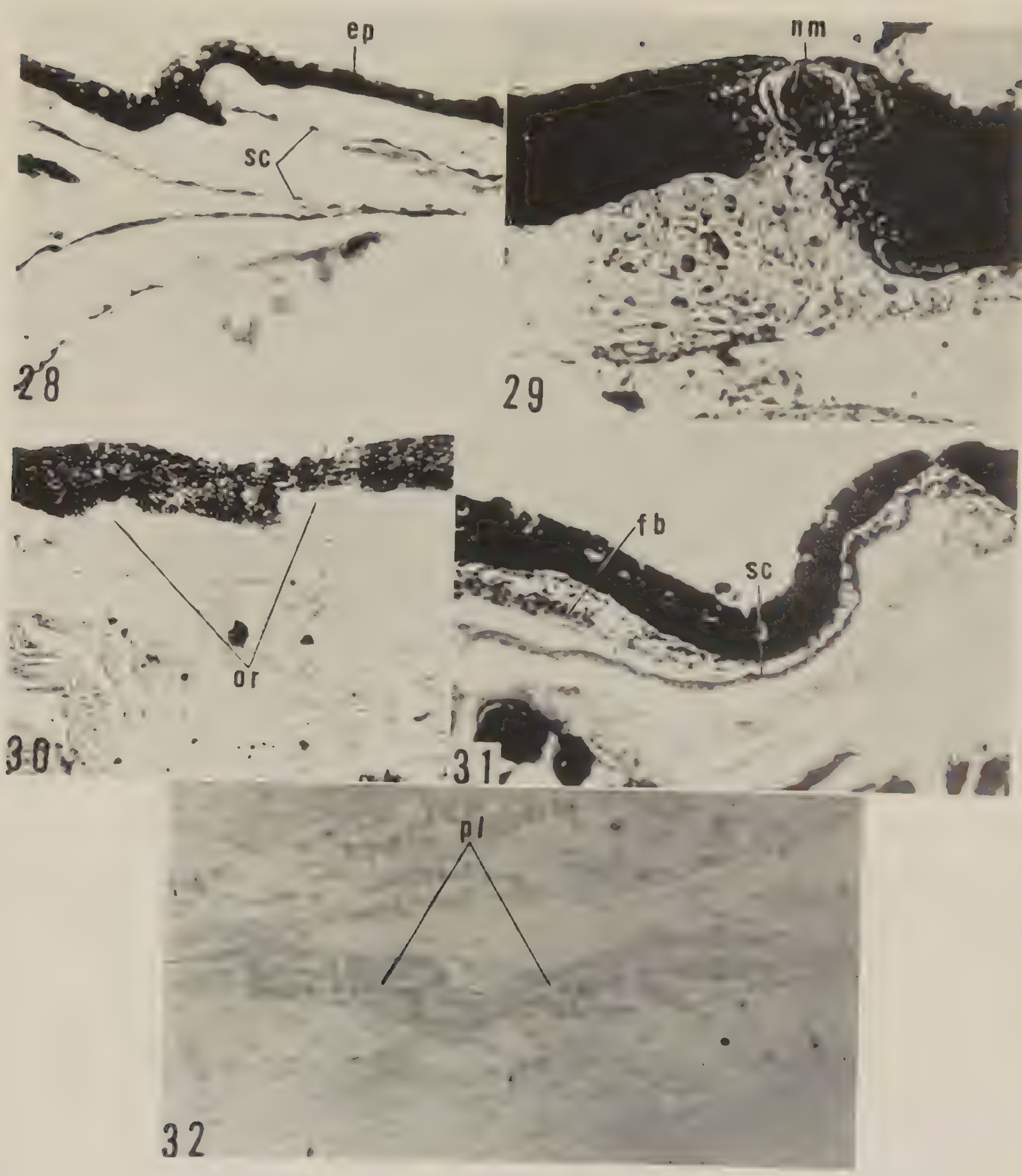
Fig. 28. Section through the ontogenetic scales. Positive alkaline phosphatase reaction is seen only in the epithelium. Gomori. ×80 ep: epithelium; sc: ontogenetic scales

Fig. 29. Section through the skin of a goldfish reared in CS, after 26 days of the removal of ontogenetic scale. Gomori. ×480 nm: neuromast

Fig. 30. Section through the skin of a goldfish reared in CF, after 78 days of the removal of ontogenetic scale. Gomori. ×100 cr: sites wherein original scales were situated

Fig. 31. Section through a regenerating scale of a goldfish, reared 12 days in WF after reared 86 days in CF. Gomori. ×150 fb: fibroblasts; sc: regenerating scale

Fig. 32. Section through the focal area of regenerating scale. Methyl green-pyronin. ×400 pl: platelets of regenerating scale



J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XIII

Fig. 33. The ordinary scale and two stages of the transplanted scale in fish No. 1 described in Table 7. ×18

ab Transplanted scale (b) placed upon the ordinary scale (a).

c Transplanted scale at 188 days after transplantation.



33 ab



C

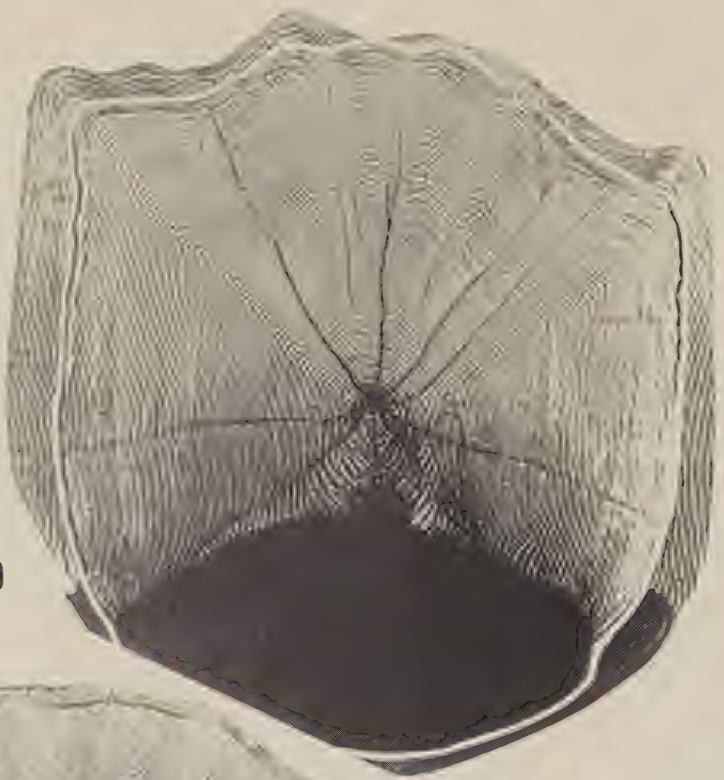
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XIV

Fig. 34. The ordinary scale and two stages of the transplanted scale in fish No. 2 described in Table 7. ×18

ab Transplanted scale (b) placed upon the ordinary scale (a).

c Transplanted scale at 188 days after transplantation.



34 ab



c

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XV

Fig. 35. The ordinary scale and two stages of the transplanted scale in fish No. 3 described in Table 7. ×18

ab Transplanted scale (b) placed upon the ordinary scale (a).

c Transplanted scale at 128 days after transplantation.



35 a b

c

J. Yamada: Studies on the Structure and Growth of the Scales

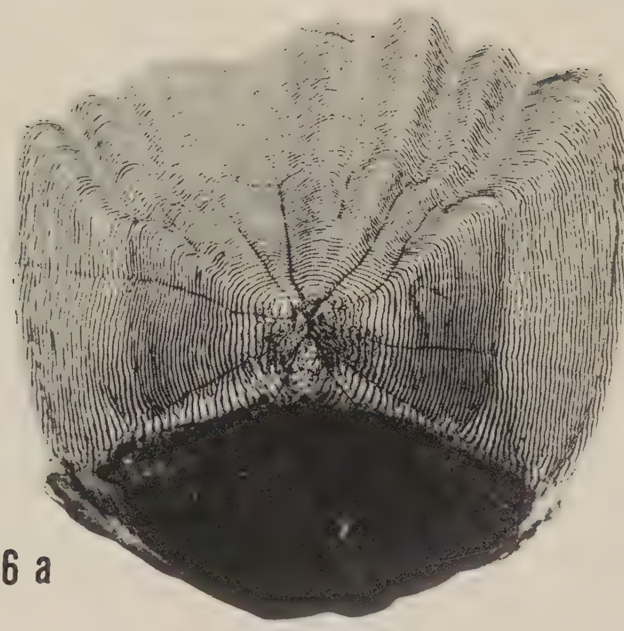
PLATE XVI

Fig. 36. The ordinary scale and two stages of the transplanted scale in fish No. 4 described in Table 7. ×13

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 73 days after transplantation.



36 a



b



c

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XVII

Fig. 37. The ordinary scale and two stages of the transplanted scale in fish No. 5 described in Table 7. ×18

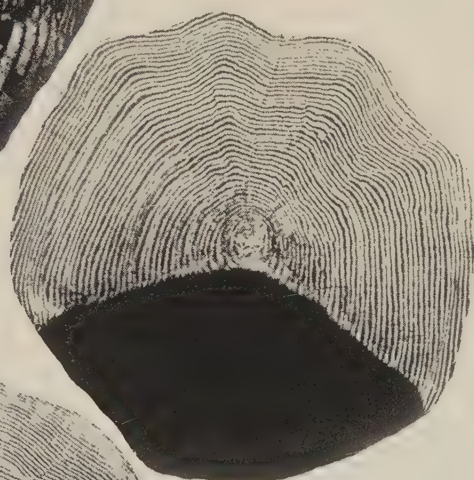
a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 187 days after transplantation.



37 a



b



c

J. Yamada: Studies on the Structure and Growth of the Scales

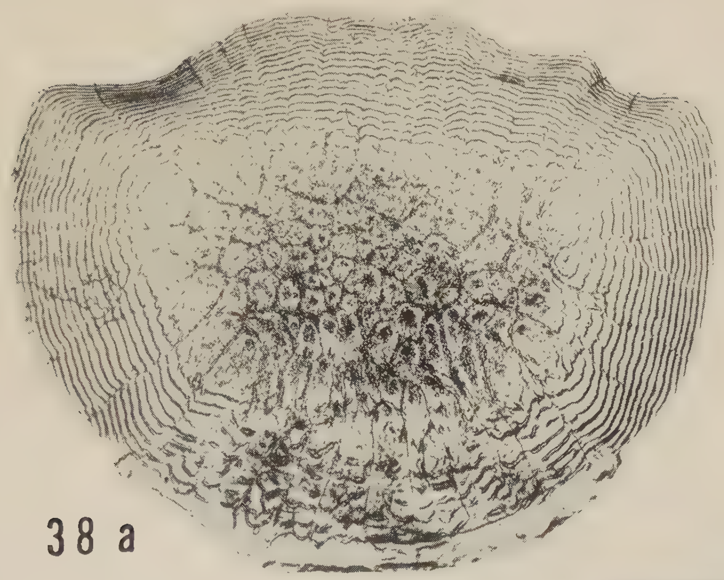
PLATE XVIII

Fig. 38. The ordinary scale and two stages of the transplanted scale in fish No. 6 described in Table 7. ×13

a Ordinary scale.

b Transplanted scale.

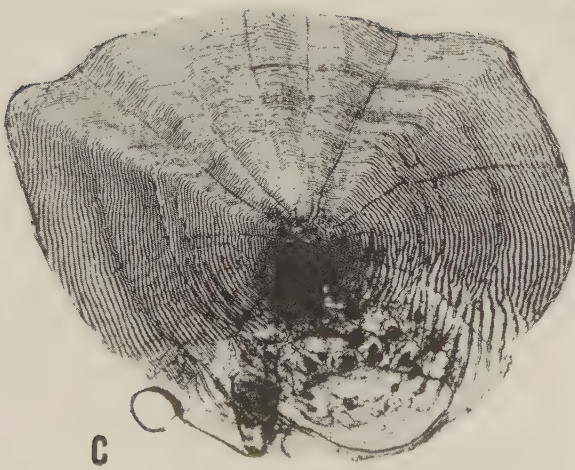
c Transplanted scale at 71 days after transplantation.



38 a



b



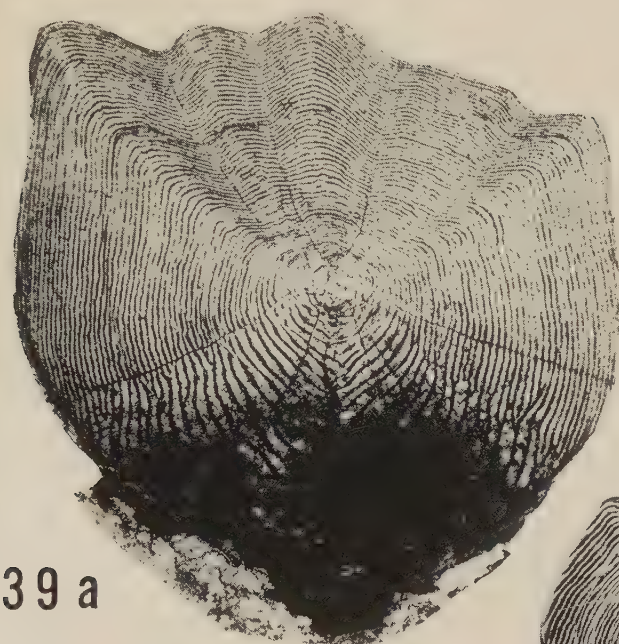
c

J. Yamada: Studies on the Structure and Growth of the Scales

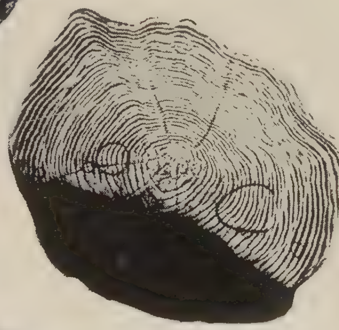
PLATE XIX

Fig. 39. The ordinary scale and two stages of the transplanted scale in fish No. 7 described in Table 7. ×18

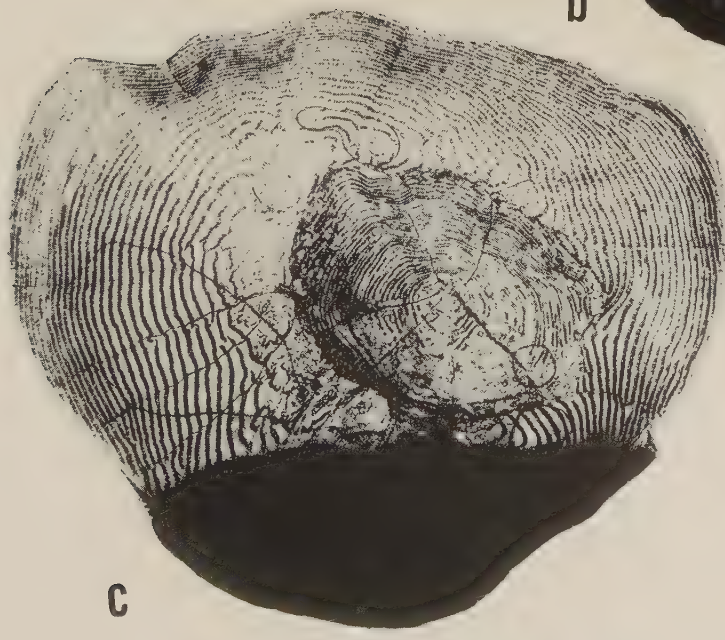
- a Ordinary scale.
- b Transplanted scale.
- c Transplanted scale at 138 days after transplantation.



39 a



b



c

J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XX

Fig. 40. The ordinary scale and two stages of the transplanted scale in fish No. 8 described in Table 7. ×18

- a Ordinary scale.
- b Transplanted scale.
- c Transplanted scale at 138 days after transplantation.



J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XXI

Fig. 41. The ordinary scale and two stages of the transplanted scale in fish No. 9 described in Table 7. ×18

a Ordinary scale.

b Transplanted scale.

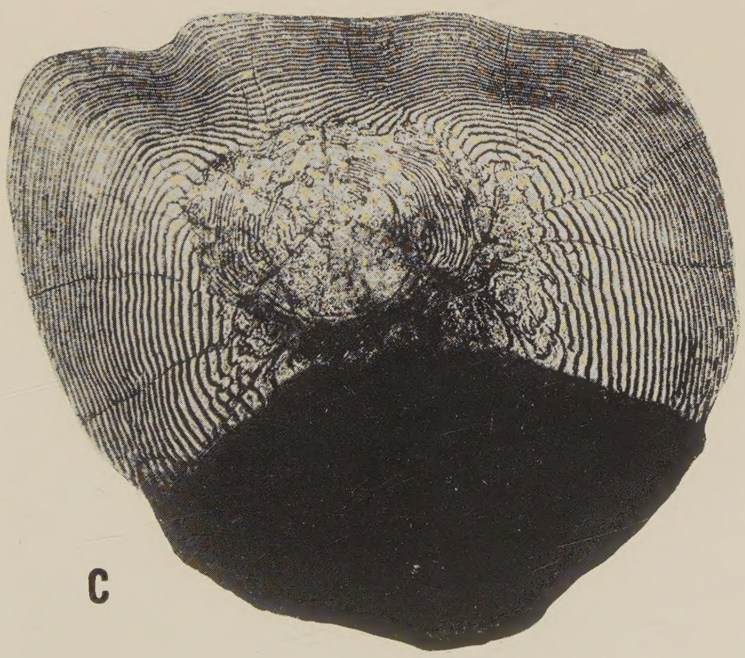
c Transplanted scale at 138 days after transplantation.



41 a



b



c

J. Yamada: Studies on the Structure and Growth of the Scales

昭和36年11月15日　印　刷
昭和36年11月31日　発　行

北海道大学水産学部
函館市港町253番地

印刷所　株式会社　笠井出版印刷社
東京都港区芝南佐久間町1の53

MEMOIRS
OF THE
FACULTY OF FISHERIES
HOKKAIDO UNIVERSITY

Vol. 1, No. 1 (March, 1953)
MOTODA, S. — OBSERVATIONS ON DIURNAL MIGRATION OF PLANKTON CRUSTACEANS IN LAKES SHIKOTSU, HOKKAIDO, AND TSUGARUJUNI, AOMORI, AND SOME EXPERIMENTS ON PHOTO-AND GEOTROPISM

Vol. 2, No. 1 (December, 1954)
TOKIDA, J. — THE MARINE ALGAE OF SOUTHERN SAGHALIEN

Vol. 3, No. 1 (August, 1955)
TANIKAWA, E. — STUDIES ON THE PROTEINS OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

Vol. 3, No. 2 (December, 1955)
SUZUKI, N. — STUDIES ON THE MANUFACTURE OF ALGIN FROM BROWN ALGAE
YOSHIMURA, K. — STUDIES ON THE TROPOMYOSIN OF SQUID

Vol. 4, No. 1 (August, 1956)
KAWAMURA, T. — LIMNOLOGICAL INVESTIGATIONS OF THE TSUGARUJUNIKO LAKE GROUP, AOMORI PREFECTURE, NORTHERN JAPAN, WITH SPECIAL REFERENCE TO THE PLANKTON COMMUNITIES

Vol. 4, No. 2 (December, 1956)
KATO, K. — CHEMICAL INVESTIGATIONS ON MARINE HUMUS IN BOTTOM SEDIMENTS

Vol. 5, No. 1 (December, 1957)
SAITO, I. — STUDIES ON FISHING GROUND, FISHING GEARS AND FISHING TECHNIQUE IN ONE-BOAT MEDIUM TRAWL FISHERY

Vol. 5, No. 2 (December, 1957)
HAMANO, S. — PHYSICO-CHEMICAL STUDIES ON THE ACTIVATION AND FERTILIZATION OF FISH EGGS

Vol. 6, No. 1 (December, 1958)
OHMI, H. — THE SPECIES OF GRACILARIA AND GRACILARIOPSIS FROM JAPAN AND ADJACENT WATERS

Vol. 6, No. 2 (December, 1958)
TANIKAWA, E. — STUDIES ON THE TECHNICAL PROBLEMS IN THE PROCESSING OF CANNED SALMON

Vol. 7, No. 1/2 (December, 1959)
NIIYAMA, H. — A COMPARATIVE STUDY OF THE CHROMOSOMES IN DECAPODS, ISOPODS AND AMPHIPODS, WITH SOME REMARKS ON CYTOTAXONOMY AND SEX-DETERMINATION IN THE CRUSTACEA
YABU, H. & KAWAMURA, K. — CYTOLOGICAL STUDY ON SOME JAPANESE SPECIES OF RHODOMELACEAE
MOTODA, S. — DEVICES OF SIMPLE PLANKTON APPARATUS
TANIKAWA, E. — STUDIES ON TECHNICAL PROBLEMS IN THE PROCESSING OF CANNED CRAB

Vol. 8, No. 1 (November, 1960)
ISHIHARA, Y. — STUDIES ON CRYSTALLINE WHALE PEPSIN

Vol. 8, No. 2 (December, 1960)
NAGASAWA, Y. — STUDIES ON THE "BROWNING" OF CANNED CRAB MEAT (*PARALITHODES CAMTSCHATICA* TIL.)

Vol. 9, No. 1 (June, 1961)
HAMADA, K. — TAXONOMIC AND ECOLOGICAL STUDIES OF THE GENUS *HYPOMESUS* OF JAPAN
KUBO, S. — SQUID TROPOMYOSINS

Vol. 9, No. 2 (November, 1961)
AKIBA, M. — STUDIES ON BOUND WATER IN FISH MUSCLE
YAMADA, J. — STUDIES ON THE STRUCTURE AND GROWTH OF THE SCALES IN THE GOLDFISH